Microbial Electrolysis Cells for production of methane from CO$_2$: long-term performance and perspectives

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1   ABSTRACT.

2   A methane producing Microbial Electrolysis Cell (MEC) is a technology to convert CO_2 into

3   methane, using electricity as an energy source and microorganisms as the catalyst. A methane

4   producing MEC provides the possibility to increase the fuel yield per hectare of land area, when the

5   CO_2 produced in biofuel production processes is converted to additional fuel methane. Besides

6   increasing fuel yield per hectare of land area, this also results in more efficient use of land area,

7   water, and nutrients. In this research, the performance of a methane producing MEC was studied for

8   188 days in a flat plate MEC design. Methane production rate and energy efficiency of the methane

9   producing MEC were investigated with time to elucidate the main bottlenecks limiting system

10   performance. Using water as the electron donor at the anode during continuous operation, methane

11   production rate was 0.006 m^3/m^3 per day at a cathode potential of -0.55 V vs. NHE with a coulombic

12   efficiency of 23.1%. External electrical energy input was 73.5 kWh/m^3 methane, resulting in a

13   voltage efficiency of 13.4%. Consequently, overall energy efficiency was 3.1%. The maximum

14   achieved energy efficiency was obtained in a yield test and was 51.3%. Analysis of internal

15   resistance showed that on the short-term, cathode and anode losses were dominant, but with time,

16   also pH gradient and transport losses became more important. The results obtained in this study are

17   used to discuss the possible contribution of methane producing MECs to increase in fuel yield per

18   hectare of land area.

19

20   KEY WORDS

21   Microbial Electrolysis Cell, CO_2 reduction, methane, biomass efficiency, land use

22

1   **Introduction**

2   There is a societal need for the production of fuels from alternative, renewable energy sources to

3   substitute fossil fuels. Organic material (biomass) is an attractive feedstock for the production of

4   biofuels, as it is often locally available, it could contribute to reducing greenhouse gas emissions

5   when produced and utilized in a sustainable way, and biomass can be easily stored [1]. The current

6   biofuel debate, however, shows that it is questionable whether sufficient biomass can be produced in

7   a sustainable way to cover all the world's fuel needs [2, 3]. To achieve a higher and more sustainable

8   biofuel production, it is therefore of importance that the fuel yield per hectare of the available land

9   area should be increased, while water and nutrients should be used as efficiently as possible [4, 5].

10   The amount of biomass used for energy production has increased considerably from 648 Mtoe in

11   1973 to 1179 Mtoe in 2007 [6]. In the conversion of biomass into biofuel, e.g. fermentation of sugars

12   into ethanol or anaerobic digestion of acetate into methane, most of the oxygen atoms present in

13   organic material need to be removed to produce a high energy density fuel. These oxygen atoms are

14   removed in the form of CO_2 [7]. For example, in case of fermentation of sugars into ethanol, for

15   each mole of ethanol produced, 1 mole of CO_2 is formed. Similarly, in case of anaerobic digestion of

16   acetate into methane, for each mole of methane produced, 1 mole of CO_2 is formed. In this process

17   of removing oxygen atoms in the form of CO_2 part of the carbon present in biomass is lost [7]. If the

18   by-product CO_2 is converted into additional fuel, this would increase the fuel yield per hectare of

19   land area. This increases not only the fuel yield land use efficiency, but also the efficiency of use of

20   nutrients and water. This research therefore investigates whether a Microbial Electrolysis Cell

21   (MEC) is a suitable technology to convert CO_2 to additional fuel.

22   An MEC is a novel technology that uses renewable electricity as the energy source for the

23   production of fuels and chemicals, such as hydrogen [8], ethanol [9], and hydrogen peroxide [10]. A

24   recently developed application for MECs is to produce methane from CO_2 using microorganisms as

25   the catalyst, with input of electrical energy [11]. A methane producing MEC consists of two

26   electrodes, anode and cathode, separated by a membrane. The membrane is essential to produce pure

27   products [12]. At the anode, an oxidation reaction takes place, e.g. the oxidation of acetate or water,

yielding CO_2 or O_2 respectively, and protons and electrons. Electrons are released to the anode and flow through an external electrical circuit to the cathode, while protons and cations migrate through the membrane to the cathode to maintain electroneutrality. At the cathode, the protons and electrons are used to produce methane. The reaction at the cathode is catalyzed by electrochemically active microorganisms, i.e. hydrogenotrophic methanogens [11], and the cathode is therefore called a biocathode. In a methane producing MEC, the overall reaction is thermodynamically not favorable and needs electrical energy to drive the reaction [13].

Hydrogenotrophic methanogens can catalyze methane production from CO_2 in an MEC via two mechanisms: (i) direct extracellular electron transfer (eq. 1), i.e. the electrons are directly taken up from the electrode and used to reduce the CO_2 to methane,

$$CO_2 + 8\ H^+ + 8\ e^- \rightarrow CH_4 + 2\ H_2O \qquad (E_{cat} = -0.24\ V\ vs.\ NHE\ [11]) \qquad (1)$$
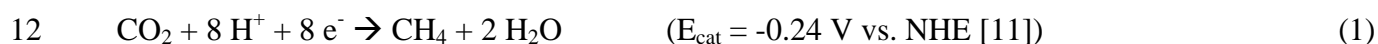
All reported potentials are standard potentials under biological relevant conditions at pH 7 and 25°C.

and (ii) indirect extracellular electron transfer (eq. 2 and 3), i.e. with intermediate production of hydrogen [11, 14]. In this mechanism first hydrogen is produced at the cathode either electrochemically or bio-electrochemically (eq. 2).

$$2\ H^+ + 2\ e^- \rightarrow H_2 \qquad (E_{cat} = -0.41\ V\ vs.\ NHE\ [15]) \qquad (2)$$
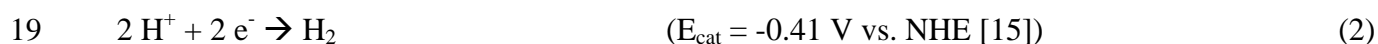
This hydrogen is used together with CO_2 to produce methane (eq. 3).

$$CO_2 + 4\ H_2 \rightarrow CH_4 + 2\ H_2O \qquad (3)$$

Methane production via direct extracellular electron transfer is considered the most energy-efficient process, as the standard potential of hydrogen production via indirect extracellular electron transfer ($E_{cat} = -0.41$ V vs. NHE) is lower than of methane production via direct extracellular

1   electron transfer (E_{cat} = -0.24 V vs. NHE). The protons and electrons needed for the reduction

2   reaction at the cathode are produced by oxidizing water at the anode (eq. 4).

3

4         $4 H_2O \rightarrow 2 O_2 + 8 H^+ + 8 e^-$ (E_{an} = 0.81 V vs. NHE [13])   (4)

5

6   The overall process is the production of methane from CO_2 and water (eq. 5).

7

8         $CO_2 + 2 H_2O \rightarrow CH_4 + 2 O_2$ (E_{cell} = -1.05 to -1.22 V vs. NHE)   (5)

9

10   The minimum thermodynamic energy input required is thus achieved when employing direct

11   reduction of CO_2 to methane, and is 32.7 MJ electrical energy per m^3 of methane (9.1 kWh/m^3

12   methane) under biologically relevant conditions (pH 7 and 25˚C).

13   This paper studies whether a methane producing MEC is a suitable technology for increasing the

14   fuel yield per hectare of land area. Previous studies on methane producing MECs focused on the

15   mechanism of methane production and showed that direct reduction of CO_2 to methane coupled to

16   water oxidation is feasible with an additional energy input on top of the thermodynamic energy input

17   [11, 14]. However, the different types of energy losses occurring in the MEC that lead to this

18   additional energy input were not extensively studied. Insight in these energy losses is essential to

19   improve the performance of methane producing MECs, and consequently to increase the fuel yield

20   per hectare of land area. This study therefore investigated the nature and extent of energy losses in a

21   methane producing MEC and their effects on MEC energy efficiency and methane production rates.

22   The oxygen produced at the anode could negatively affect the performance of methane producing

23   MECs. The oxygen might diffuse to the cathode [16] and could lead to parasitic reactions either via

24   direct reduction to water at the cathode or via oxidizing the methane produced. Oxygen is also

25   known to inhibit hydrogenotrophic methanogens [17] and might decrease the methane production

26   rate. To understand a possible negative role of oxygen, the methane producing MEC was first

27   operated for 83 days using hexacyanoferrate(II) oxidation at the anode and subsequently for 105

1 days using water oxidation at the anode. The performance of the biocathode in both periods was

2 analyzed using polarization curves and yield tests. The overall performance of the methane

3 producing MEC was determined by analyzing the resistances of the different elements of the

4 methane producing MEC, i.e. anode, cathode, membrane, and electrolyte, and by analyzing the

5 overall energy efficiency. The results obtained in this study were finally used to discuss the possible

6 contribution of methane producing MECs to increase in fuel yield per hectare of land area.

7

8 **Materials and methods**

9 *Experimental set-up*

10 The experiment was performed in the same electrochemical cell as described in Sleutels *et al.* [18]

11 with a total volume of 0.56 L using a cation exchange membrane (0.7 mm, Ralex CM, Mega a.s.,

12 Czech Republic). The anode was made of platinum coated (50 g/m^2) titanium mesh (projected

13 surface area 250 cm^2, thickness 1 mm, specific surface area 1.7 m^2/m^2 – Magneto Special Anodes

14 BV, The Netherlands). The cathode was made of graphite felt (projected surface area 0.025 m^2,

15 thickness 3 mm – FMI Composites Ltd., Scotland). Both the anode and cathode compartment were

16 equipped with an Ag/AgCl 3 M KCl reference electrode (+0.205 V vs. NHE; ProSense QiS, The

17 Netherlands). The electrochemical cell was connected to a PC via a Fieldpoint FP-AI-110 module

18 (National Instruments, United States) and every 60 seconds cell voltage, current, and cathode and

19 anode potential were recorded using LabVIEW 7.1 (National Instruments, United States). A

20 luminescent dissolved oxygen probe (LDO10101, Hach, USA) was installed in the catholyte, and

21 every 30 minutes dissolved oxygen in the catholyte was measured. The system was operated in a

22 temperature controlled chamber at 30ºC.

23

24 *Electrolytes and microorganisms*

25 Oxygen, product of water oxidation at the anode, can possibly affect methanogens at the cathode

26 [17]. To prevent oxygen to affect biofilm development at the cathode at the start of the experiment,

27 hexacyanoferrate(II) was oxidized at the anode. Because hexacyanoferrate(II) is not sustainable as it

is not self-regenerating [13], it was changed to water oxidation on day 83. At start, anolyte consisted of 100 mM potassium hexacyanoferrate(II) and was circulated at 1.5 mL/s. The anolyte was refreshed regularly to avoid depletion of electron donor. The anolyte was changed to demineralized water containing 20 mM potassium phosphate buffer on day 83. The catholyte influent consisted of a 20 mM potassium phosphate buffer, macronutrients (280 mg/L NH_4Cl, 5.7 mg/L $CaCl_2$, 10 mg/L $MgSO_4 \cdot 7H_2O$, 90 mg/L $MgCl_2 \cdot 6H_2O$), and 1 mL/L of a micronutrients and vitamin solution same as [19]. The catholyte influent was supplemented with 5 g/L $NaHCO_3$ as carbon source, as at operating pH 7 CO_2 is mainly present as HCO_3^-, and circulated at 1.5 mL/s. The catholyte was continuously refreshed at a rate of 0.1 mL/min (Stepdos 03RC, KNF, Germany). 5 gram of anaerobic sludge, obtained from an UASB treating distillery wastewater (Nedalco, The Netherlands), was used as inoculum and the electrochemical cell was flushed with pure nitrogen (>99.9992%) for 30 minutes before applying a cell voltage. pH of the catholyte was controlled at pH 7±0.1 through a pH controller (Liquisis M CPM 253, Endress+Hauser, Switzerland) using 1 M HCl.

MEC operation

During long-term MEC operation there is a risk on malfunctioning of the reference electrodes in the electrochemical cell. To prevent damage to the methane producing biocathode as a result of malfunctioning of the reference electrode, cell voltage was controlled instead of cathode potential. The electrochemical cell was connected to a power supply (MCP94, Bank Elektronik, Germany). Cell voltage was adjusted to obtain the desired cathode potential, when cathode potential deviated >20 mV from the desired cathode potential.

The experiment was started using a biocathode from an already running MEC using hexacyanoferrate(II) oxidation at the anode. This biocathode had a current density of 0.3 A/m^2 and was operated at an E_{cat} of -0.8 V vs. NHE. At the start of the experiment (day 0) this biocathode was inoculated to make sure that sufficient biomass was present. From day 0 to day 83, with hexacyanoferrate(II) as the anolyte, the cathode potential was controlled at -0.7 V vs. NHE. After switching the anolyte to water (day 83 to 177), the cathode potential was -0.55 V vs. NHE. This

1   potential was higher than with hexacyanoferrate(II), and is the result of the maximum range in

2   applied cell voltage of the potentiostat, which was limited to -2 V. This range limited the cathode

3   potential as the anode potential required for water oxidation was higher than the anode potential

4   required for hexacyanoferrate(II) oxidation. On day 188, the experiment was finished due to

5   leakages in the MEC.

6

7   *Polarization tests*

8   Polarization tests were made using an IviumStat potentiostat with a Peripheral Port Expander

9   (Ivium Technologies, Eindhoven, The Netherlands). The applied cathode potential was decreased

10   from -0.4 V to -1.0 V with steps of 0.1 V, each step lasting 10 minutes, using hexacyanoferrate(II)

11   oxidation at the anode. When using water oxidation at the anode, the cell voltage was controlled

12   instead of cathode potential. In this case, the applied cell voltage was decreased from -1.0 V to -2.0

13   V with steps of 0.1 V, each step lasting 10 minutes, and cathode potential was continuously recorded

14   versus a reference electrode. This resulted in a cathode potential at highest -0.21 V vs. NHE and at

15   lowest -0.78 V vs. NHE using water oxidation at the anode. Current was recorded each second and

16   the last ten data points at each cathode potential were averaged and plotted in the polarization curve.

17

18   *Gas analysis*

19   Gas composition of the cathode gas phase was measured with two different gas chromatographs,

20   same as [20], to measure all gasses present. Gas production was measured with a gas flow meter

21   (Milligascounter®, Ritter, Germany).

22   Methane production rate was calculated from the measured gas production and gas composition

23   using the mass balance equation described by [21]:

24
$$V_{CH_{4,t}} = V_{CH_{4,t-1}} + (V_{T,t} - V_{T,t-1}) \cdot \frac{c_{CH_{4,t}} + c_{CH_{4,t-1}}}{2} + V_{cat} \cdot (c_{CH_{4,t}} - c_{CH_{4,t-1}})$$
(6)

25   with $V_{CH4,t}$ and $V_{CH4,t-1}$ the cumulative methane gas production (L CH_4) on sample time t and

26   previous sample time t-1, respectively, $V_{T,t}$ and $V_{T,t-1}$ the total gas production measured with a gas

27   flow meter (L) on sample time t and previous sample time t-1, respectively, $C_{CH4,t}$ and $C_{CH4,t-1}$ the

1 measured methane fractions in the cathode gas phase (-) on sample time t and previous sample time

2 t-1, respectively, and V_{cat} the cathode headspace volume (0.7 L).

3

4 *Yield tests*

5 To compare performance of the methane producing biocathode between the two anolytes

6 hexacyanoferrate(II) and water, a methane yield tests of 8h was performed on day 70 (13 days before

7 switching anolytes), and day 101 (18 days after switching anolytes). Before the yield test was

8 started, the cathode compartment was flushed with pure nitrogen (>99.9992%) for 30 minutes.

9 During the yield test, the cathode potential was controlled at -0.7 V vs. NHE using an IviumStat

10 potentiostat. Catholyte was continuously refreshed as described for continuous operation, and at the

11 start and end of the yield test the cathode gas phase was analyzed for methane as described.

12

13 *Energy efficiency*

14 The two most important parameters to describe performance of the MEC are methane production

15 rate and energy efficiency. Methane production rate was determined as described in yield tests.

16 Energy efficiency (eq. 7) of a methane producing MEC is the product of coulombic efficiency (eq. 8)

17 and voltage efficiency (eq. 9) [12].

18
$$\eta_{energy} = \eta_{CE} \cdot \eta_{voltage} = \frac{-\Delta G_{CH_4} \cdot V_{CH_4}}{V_m \cdot E_{cell} \cdot \int\limits_{t=0}^{t} I dt} \tag{7}$$

19 Coulombic efficiency (η_{CE}, %), the efficiency of capturing the electrons from the electric current

20 in methane, was calculated via

21
$$\eta_{CE} = \frac{V_{CH_4} \cdot F \cdot n}{V_m \cdot \int\limits_{t=0}^{t} I dt} \tag{8,}$$

22 with V_{CH_4} the cumulative methane gas production (m^3 CH_4), F the Faradays constant (96485

23 C/mole e⁻), n the moles of electrons per mole of methane (8 mole e⁻/mole CH_4), V_m the molar

24 volume (0.0252 m^3/mole), I the current (A), and t the time (s).

Voltage efficiency, the amount of external electrical energy that ends up in methane, was calculated via the Gibb's free energy of oxidation of methane over the electrical energy input of the MEC

$$\eta_{voltage} = \frac{E_{emf}}{E_{cell}} = \frac{-\Delta G_{CH_4}}{E_{cell} \cdot n \cdot F} \qquad (9),$$

with E_{emf} the so called reversible energy loss (V), i.e. the electrical energy converted into chemical energy in the form of methane, E_{cell} the energy input of the MEC, i.e. the applied cell voltage (V), and ΔG_{CH4} the Gibb's free energy of oxidation of methane (890.4 kJ/mole CH_4 [22]).

To improve the performance of the methane producing MEC, factors that affect the energy efficiency should be identified. Therefore, the effect of oxygen diffusion through the membrane on MEC performance was calculated, and an internal resistance analysis of the methane producing MEC was performed as described hereafter.

Oxygen diffusion through membrane

The coulombic efficiency reflects to which extent other electron consuming reactions, so called parasitic reactions, than the preferred CO_2 reduction reaction take place in the electrochemical cell. Parasitic oxidation occurs when oxygen produced at the anode diffuses to the cathode, where it oxidizes methane and/or hydrogen to CO_2 and/or water. This results in a decrease in methane production rate and a lower coulombic efficiency (smaller part of the electrons ending up in methane). Parasitic reduction occurs when oxygen is directly reduced to water at the cathode. The contribution of parasitic oxidation or reduction to coulombic efficiency can be estimated by calculating the oxygen diffusion flux (J_{O2} in mole O_2/m^2 per second) over the cation exchange membrane using Fick's law

$$J_{O_2} = D_{O_2} \cdot \frac{(c_{O_{2,an}} - c_{O_{2,cat}})}{\delta} \qquad (10)$$

with D_{O2} the diffusion coefficient of oxygen determined for a CMI-7000 cation exchange membrane (Membrane International Inc., USA) ($4.3 \cdot 10^{-10}$ m^2/s) [16], $C_{O2,an}$ and $C_{O2,cat}$ the dissolved oxygen concentration at the anode and cathode (mole O_2/m^3), respectively, and δ the thickness of the

1 membrane as supplied by the manufacturer ($0.7 \cdot 10^{-3}$ m). The oxygen diffusion flux is inversely

2 proportional to the thickness of the membrane. The estimated rate of methane oxidation ($r_{CH4,ox}$ in m^3

3 CH_4/m^3 per day) due to this flux of oxygen is

4
$$r_{CH_{4,ox}} = \frac{J_{O_2} \cdot A \cdot V_m \cdot t}{2 \cdot V_{reactor}}$$
(11)

5 with A the membrane surface area (0.025 m^2), t the time (d), $V_{reactor}$ the total reactor volume (0.56

6 L), and taking into account that 2 moles of oxygen are consumed per mole methane.

7

8 *Internal resistance analysis*

9 The applied cell voltage consists of a thermodynamically calculated cell voltage needed to produce

10 methane under biologically relevant conditions, the reversible energy loss E_{emf}, and internal energy

11 losses, the so called irreversible energy losses [18]. Irreversible energy losses, energy lost as a result

12 of the resistances of different parts of the MEC, consist of the pH gradient over the membrane

13 ($E_{\Delta pH}$), cathode overpotential (η_{cat}), anode overpotential (η_{an}), ionic losses (E_{ionic}), and transport

14 losses (E_T), that were calculated according to [18]

15
$$E_{cell} = E_{emf} - E_{\Delta pH} - \eta_{cat} - \eta_{an} - E_{ionic} - E_T$$
(12)

16 with $E_{emf} = E_{cat} - E_{an}$ (V). Cathode (E_{cat}) and anode (E_{an}) potential were calculated using the Nernst

17 equation under experimentally relevant conditions ($E^0_{Fe(II)} = 0.361$ V vs. NHE, T = 303 K, [Fe(CN)$_6^{4-}$

18] is assumed on average to be equal to [Fe(CN)$_6^{3-}$] [13], $E^0_{H_2O} = 1.229$ V vs. NHE, $pO_2 = 0.28 \cdot 10^5$ Pa

19 (average measured liquid dissolved oxygen concentration cathode was 12.4 mg O_2/L (day 83-177)),

20 $E^0_{cat} = 0.169$ V vs. NHE, $pCH_4 = 0.075 \cdot 10^5$ Pa (pressure cathode compartment was 1.005 bar with an

21 average measured methane concentration of 8.78% (day 0-188)), [HCO$_3^-$] = 0.06 M, [H$^+$] = 10^{-7} M).

22 As anolyte and catholyte pH changed with time, these were included in the irreversible energy losses

23 calculations.

24
$$E_{an,Fe(II)} = E^0_{Fe(II)} - \frac{R \cdot T}{F} \cdot \ln(\frac{[Fe(CN)_6^{4-}]}{[Fe(CN)_6^{3-}]}) = 0.36V \text{ vs. NHE}$$
(13)

$$E_{an,H_2O} = E_{H_2O}^0 - \frac{R \cdot T}{4 \cdot F} \cdot \ln(\frac{1}{pO_2 \cdot [H^+]^4}) = 0.87V \text{ vs. NHE} \qquad (14)$$

$$E_{cat} = E_{cat}^0 - \frac{R \cdot T}{8 \cdot F} \cdot \ln(\frac{[CH_4]_{sat}}{[HCO_3^-] \cdot [H^+]^9}) = -0.34V \text{ vs. NHE} \qquad (15)$$

Ionic and transport losses were calculated from all other potential losses using equation 12. Parameters measured in time to calculate potential losses were anode and cathode potential, catholyte and anolyte pH, and cell voltage. At a constant applied cell voltage, the current density that is produced by MECs depends on the internal resistance of the MEC. Partial resistances (R_i in $\Omega \cdot m^2$) were calculated by dividing the calculated potential loss by the current density, and total internal resistance was the sum of the partial resistances [18].

For analysis of internal resistance during continuous operation, six representative periods were chosen. These six periods were:

1) at the start of the experiment using hexacyanoferrate(II) oxidation (days 2-6, indicated as 'start Fe'),

2) in the middle of stable operation using hexacyanoferrate(II) oxidation (days 50-57, indicated as 'middle Fe'),

3) before switching anolytes (day 83, indicated as 'end Fe'),

4) after switching anolytes (day 83, indicated as 'start water'),

5) in the middle of stable operation using water oxidation (days 125-132, indicated as 'middle water'), and

6) at the end of the experiment using water oxidation (days 167-176, indicated as 'end water').

1   Results and discussion
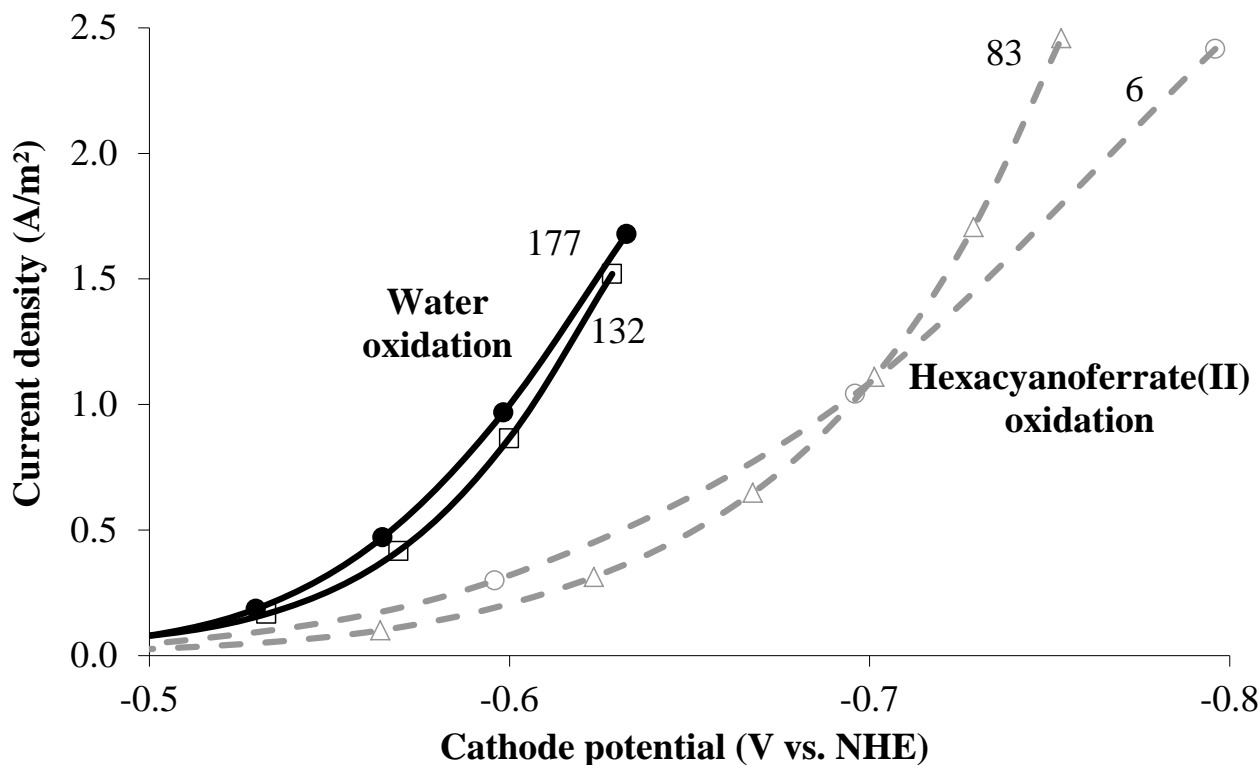
Figure 1. Polarization tests give insight in the performance of a methane producing biocathode in time (operation day indicated in figure) using hexacyanoferrate(II) (grey dashed line) or water (black solid line) oxidation at the anode.

Biocathode performance

Polarization tests are a useful tool to give insight in the development of the methane producing biocathode with time. A higher current density at the same cathode potential indicates an increase in performance. When using hexacyanoferrate (day 0-83) at the anode, during continuous operation cathode potential was controlled at -0.7 V vs. NHE, and current density was on average 0.78 ± 0.20 A/m^2. The polarization curves as shown in Figure 1 show that the biocathode had similar performance on days 6 and 83, indicating stable performance. The current density obtained in the polarization curves at -0.7V vs. NHE is well within the range obtained during continuous operation. After changing the anode reaction to water oxidation and increasing the cathode potential to -0.55 V vs. NHE during continuous operation, current density was on average 0.25 ± 0.04 A/m^2. Similar to using hexacyanoferrate(II) oxidation at the anode, the polarization curves show that the biocathode

13

1   had similar performance on days 132 and 177, indicating stable performance, and the current density

2   obtained in the polarization curves at -0.55 V vs. NHE is in line with the current density obtained

3   during continuous operation.

4   Figure 1 also shows that at the same cathode potential the biocathode performed better when using

5   water oxidation compared to hexacyanoferrate(II) oxidation. The higher current density using water

6   oxidation compared to hexacyanoferrate(II) oxidation could be the result of oxygen diffusion over

7   the membrane resulting in parasitic reactions at the cathode, or the result of better performance of

8   the biomass on the biocathode. Presence of oxygen at the cathode could lead to increased current

9   production through direct oxygen reduction at the cathode. Whether parasitic reactions at the cathode

10   are a plausible explanation for the better performance using water oxidation can be verified by

11   calculating the oxygen flux over the cation exchange membrane (eq. 10 and 11). Dissolved oxygen

12   concentration at the anode was on average 0.95 mg/L using hexacyanoferrate(II) oxidation, and 12.4

13   mg/L using water oxidation, while dissolved oxygen concentration at the cathode was always 0

14   mg/L. This leads to an oxygen flux over the membrane of $1.8 \cdot 10^{-8}$ mole O_2/m^2 per second using

15   hexacyanoferrate(II) oxidation, and $2.4 \cdot 10^{-7}$ mole O_2/m^2 per second using water oxidation. This flux

16   of oxygen can consume electrons at a rate of 0.007 A/m^2 using hexacyanoferrate(II) oxidation, and

17   0.09 A/m^2 using water oxidation. This is equal to 0.6% of the measured current density at cathode

18   potential -0.7 V vs. NHE during the polarization test using hexacyanoferrate(II) oxidation and 37%

19   of the measured current density at cathode potential -0.55 V vs. NHE during the polarization test

20   using water oxidation (Figure 1). These calculations show that oxygen diffusion over the membrane

21   can explain part of the higher current density using water oxidation.

22   After correction for the additional current density caused by possible oxygen diffusion over the

23   membrane, current density using water oxidation was still higher than when using

24   hexacyanoferrate(II) oxidation (Figure 1). This suggests that the higher controlled cathode potential

25   during continuous operation also lead to an increased current density. Using water oxidation, during

26   continuous operation cathode potential was -0.55 V vs. NHE, while during hexacyanoferrate(II)

27   oxidation the cathode potential was -0.7 V vs. NHE. The cathode potential of -0.55 V vs. NHE was

1 higher than used in previous studies, where potentials below -0.7 V vs. NHE were investigated [11,

2 14, 22, 23]. At this high cathode potential, direct CO_2 reduction to methane is energetically more

3 favorable than CO_2 reduction via hydrogen: eq. 1 and 2 show a higher potential for direct CO_2

4 reduction compared to CO_2 reduction via hydrogen, meaning that at -0.55 V vs. NHE more energy is

5 to be gained by the microorganisms via direct reduction of CO_2. Previous study indeed revealed that

6 at cathode potentials higher than -0.75 V vs. NHE, methane was mainly produced via direct CO_2

7 reduction, while at lower cathode potentials methane can also be produced indirectly via hydrogen

8 [14]. In this study, at a cathode potential of -0.55 V vs. NHE, direct reduction of CO_2 was the most

9 likely process, although we did not further investigate the mechanisms.

10   To further study the performance of the biocathode when using water oxidation at the anode

11 compared to hexacyanoferrate(II) oxidation, yield tests were performed, in which the cathode

12 potential was controlled at the same value of -0.7 V vs. NHE. This was done on day 70 (13 days

13 before switching anolytes) and day 101 (18 days after switching anolytes). Table 1 shows that during

14 the yield test, the current density was 40% higher when using water oxidation compared to

15 hexacyanoferrate(II) oxidation. This is in agreement to what was found during polarization tests

16 (Figure 1). Methane production rate was 30% higher when using water oxidation compared to

17 hexacyanoferrate(II) oxidation, and this was confirmed by the slightly lower coulombic efficiency

18 for water oxidation compared to hexacyanoferrate(II) oxidation in the yield tests. The coulombic

19 efficiency of hexacyanoferrate(II) oxidation in the yield test being higher than 100% could be a

20 result of biomass degradation and oxidation or storage of electrons in the microorganisms [24]. The

21 better performance of the biocathode when using water oxidation could also be a result of the lower

22 applied cathode potential in the yield test (-0.7 V vs. NHE) compared to the potential at which the

23 biofilm was acclimatized during continuous operation (-0.55 V vs. NHE). This change in cathode

24 potential could have affected the functioning of the biocathode.

25   The polarization curves and yield tests reveal that oxygen via parasitic reactions decreases the

26 coulombic efficiency of the biocathode, however, it has no obvious negative effect on the methane

27 production rate. This might be explained by the fact that the oxygen reacts away either via direct

1 reduction to water at the cathode or via oxidizing the methane produced, and therefore cannot affect

2 the methanogens present at the biocathode.

3

4 *Long-term current generation and methane production in flat plate MEC*
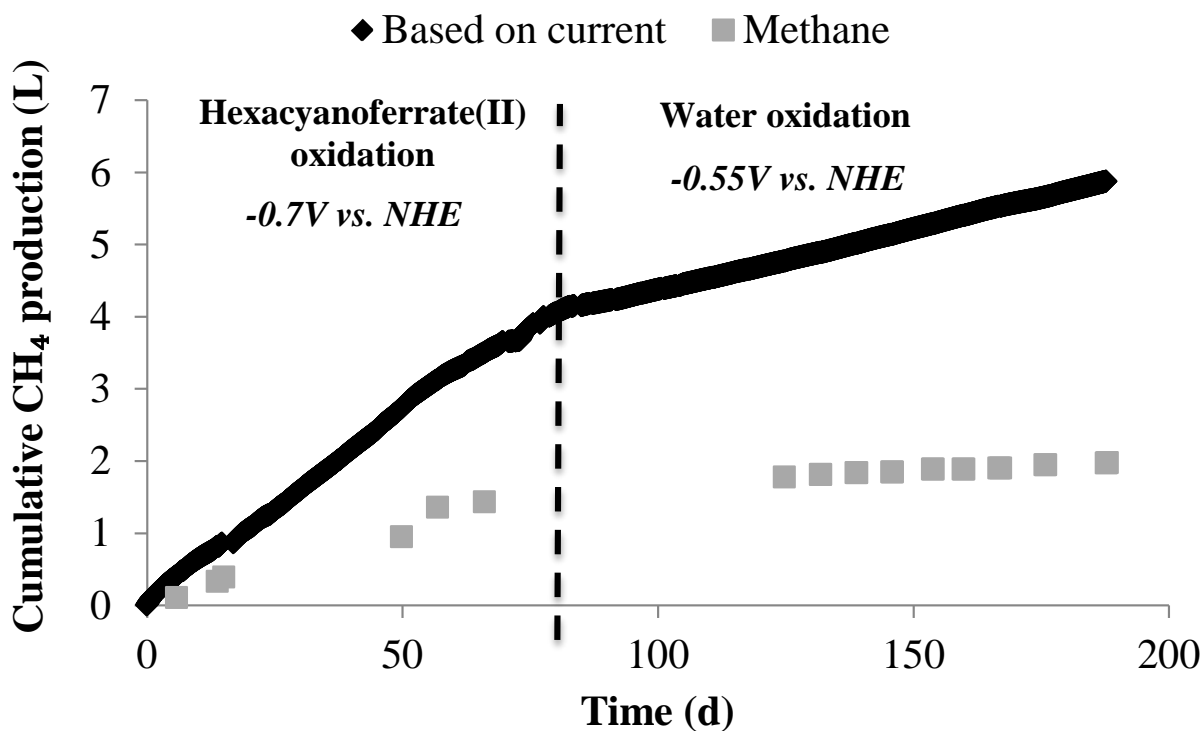
Figure 2. Cumulative methane production with time. The black diamonds indicate methane production calculated from the current, while the grey squares indicate measured methane production. Anolyte was changed from hexacyanoferrate(II) to water on day 83, as indicated by the dashed line.

11 Current and methane were produced continuously in the flat plate MEC for 188 days (Figure 2).

12 When using hexacyanoferrate (day 0-83), cathode potential was controlled at -0.7 V vs. NHE, and

13 current density ranged between 0.4 A/m^2 and 2.5 A/m^2, being on average 0.78±0.20 A/m^2. Methane

14 production ranged between 0.01-0.10 m^3 CH$_4$/m^3 per day, being on average 0.05±0.03 m^3 CH$_4$/m^3

15 per day. After changing the anode reaction to water oxidation and increasing the cathode potential to

16 -0.55 V vs. NHE, current density decreased and ranged between 0.2 A/m^2 and 0.6 A/m^2 (day 83-

17 188), being on average 0.25±0.04 A/m^2. Methane production ranged between 3·10^{-4}-0.025 m^3

1   CH_4/m^3 per day, being on average 0.006±0.008 m^3 CH_4/m^3 per day. This decrease in gas production

2   rate when switching the anolyte from hexacyanoferrate(II) to water is in line with the lower current

3   density (Figure 2).

4   Methane production rate was on average a factor 8 higher using hexacyanoferrate(II) as electron

5   donor compared to water during continuous operation (Table 1). Part of the explanation for the lower

6   methane production rate when using water oxidation is the higher cathode potential and

7   consequently the lower current density, however, this cannot be the only explanation, as the current

8   density was only a factor 3-4 lower. Parasitic reactions that consume electrons will also lead to a

9   lower methane production rate. These parasitic reactions are reflected in the coulombic efficiency,

10   which was about a factor 1.5-4 lower when using water oxidation compared to hexacyanoferrate(II)

11   oxidation during continuous operation (Table 1). Whether parasitic reactions are a plausible

12   explanation for the lower coulombic efficiency can be verified by calculating the oxygen diffusion

13   flux over the cation exchange membrane. Average dissolved oxygen concentration at the anode was

14   12.3 mg/L using water oxidation, while dissolved oxygen concentration was always 0 mg/L at the

15   cathode. This leads to an oxygen flux over the membrane of $2.4 \cdot 10^{-7}$ mole O_2/m^2 per second. This

16   flux of oxygen can oxidize methane at a rate of 0.012 m^3 CH_4/m^3 per day. If we calculate the

17   expected methane production rate using the measured methane production rate in Table 1, and taking

18   into account the methane lost due to oxidation by oxygen, then the methane production rate using

19   water oxidation was 0.005 + 0.012 = 0.017 m^3 CH_4/m^3 per day (middle water) and 0.006 + 0.012 =

20   0.018 m^3 CH_4/m^3 per day (end water). This would result in a maximum coulombic efficiency of

21   57.8% (middle water) and 67.5% (end water), which is comparable to the coulombic efficiency for

22   hexacyanoferrate(II) oxidation. These calculations show that parasitic reactions can explain part of

23   the differences in coulombic efficiency between both anolytes.

24   Parasitic reactions alone, however, cannot explain why coulombic efficiency is considerably lower

25   than 100% for both anolytes during continuous operation. It is believed that part of the methane is

26   lost due to diffusion from the cathode to the anode, similar to hydrogen losses in MECs [15, 25], and

27   therefore less methane is measured in the cathode gas phase than expected from the measured

current. The coulombic efficiency can likely be improved by using a membrane that is less permeable for gases. This results in less methane diffusion from the cathode to the anode, and less oxygen diffusion from the anode to cathode, and consequently in less parasitic reactions.

Methane production rates reported for methane producing bio-electrochemical systems using water oxidation are 0.012-0.015 m^3 CH_4/m^3 per day for two-compartment MECs at cathode potentials between -0.8 V and -0.9 V vs. NHE [11, 14]. The methane production rates during continuous operation using hexacyanoferrate(II) oxidation, 0.033-0.071 m^3 CH_4/m^3 per day, were higher than previously reported methane production rates. Methane production rates during continuous operation using water oxidation were somewhat lower, 0.005-0.006 m^3 CH_4/m^3 per day, however, this is expected as the results are obtained at a cathode potential of >0.25 V higher than used in other studies.

Voltage efficiency and energy efficiency

During continuous operation using hexacyanoferrate(II) oxidation at the anode, the energy input was 15.4 kWh/m^3 methane (start Fe; based on E_{cell} = -1.21 V) and 31.6 kWh/m^3 methane (middle Fe; based on E_{cell} = -1.23 V) (Table 1). Using water oxidation at the anode, the energy input was 97.2 kWh/m^3 methane (middle water; based on E_{cell} = -1.99 V) and 73.5 kWh/m^3 methane (end water; based on E_{cell} = -1.99 V) (Table 1). Gibb's free energy of methane oxidation is 9.8 kWh/m^3 methane [22], so voltage efficiency was 63.7% (start Fe) and 31.1% (middle Fe) using hexacyanoferrate(II) oxidation, and 10.1% (middle water) and 13.4% (end water) using water oxidation.

Energy efficiency (eq. 7) is the product of coulombic efficiency (eq. 8) and voltage efficiency (eq. 9) [12]. The energy efficiency was 42.6% (start Fe) and 10.3% (middle Fe) using hexacyanoferrate(II) oxidation, and 1.8% (middle water) and 3.1% (end water) using water oxidation (Table 1).

It should be noted that the energy efficiency using hexacyanoferrate(II) oxidation at the anode does not include the energy required for regeneration of hexacyanoferrate(II), which is essential for hexacyanoferrate(II) to be a sustainable electron donor for use in practical applications [13]. The

1   energy efficiency using hexacyanoferrate(II) oxidation at the anode, however, reveals the potential

2   performance of a methane producing biocathode using an efficient anode, and is useful to study the

3   effect of oxygen diffusion over the membrane on the performance of a methane producing MEC.

4   The yield tests show the maximum achieved voltage efficiency and energy efficiency. The energy

5   input in a yield test using hexacyanoferrate(II) oxidation was 16.6 kWh/m^3 methane (day 70),

6   leading to a voltage efficiency of 59.3% and an energy efficiency of 62.0% (Table 1). The energy

7   input in a yield test using water oxidation was 18.2 kWh/m^3 methane (day 101), leading to a voltage

8   efficiency of 53.9% and an energy efficiency of 51.3% (Table 1).

9

10   *Identifying sources of irreversible energy losses*

11   To study how energy efficiency can be improved, analysis of irreversible energy losses during

12   continuous operation of the methane producing MEC is essential. At a constant applied cell voltage,

13   the current density that is produced by MECs depends on the internal resistance of the MEC [18].

14   Therefore, partial resistances were calculated to identify which processes contributed most to the

15   total internal resistance of the MEC. These partial resistances represent cathode losses, anode losses,

16   losses due to the pH gradient over the membrane, and transport & ionic losses.

17   Figure 3 shows an increase in total internal resistance with time for both anolytes. This causes a

18   decrease in current density with time. Current density decreased from 1.15 A/m^2 to 0.30 A/m^2 (from

19   start Fe to end Fe) using hexacyanoferrate(II) oxidation, and from 0.30 A/m^2 to 0.25 A/m^2 (from

20   start water to end water) using water oxidation. From Figure 3, also two main effects can be seen: (i)

21   in case of hexacyanoferrate(II) oxidation, the total internal resistance was considerably lower than

22   with water oxidation, and was mainly caused by the cathode, and (ii) in case of water oxidation, in

23   the beginning anode and cathode contributed most to the total internal resistance, while pH and

24   transport & ionic losses increased with time.
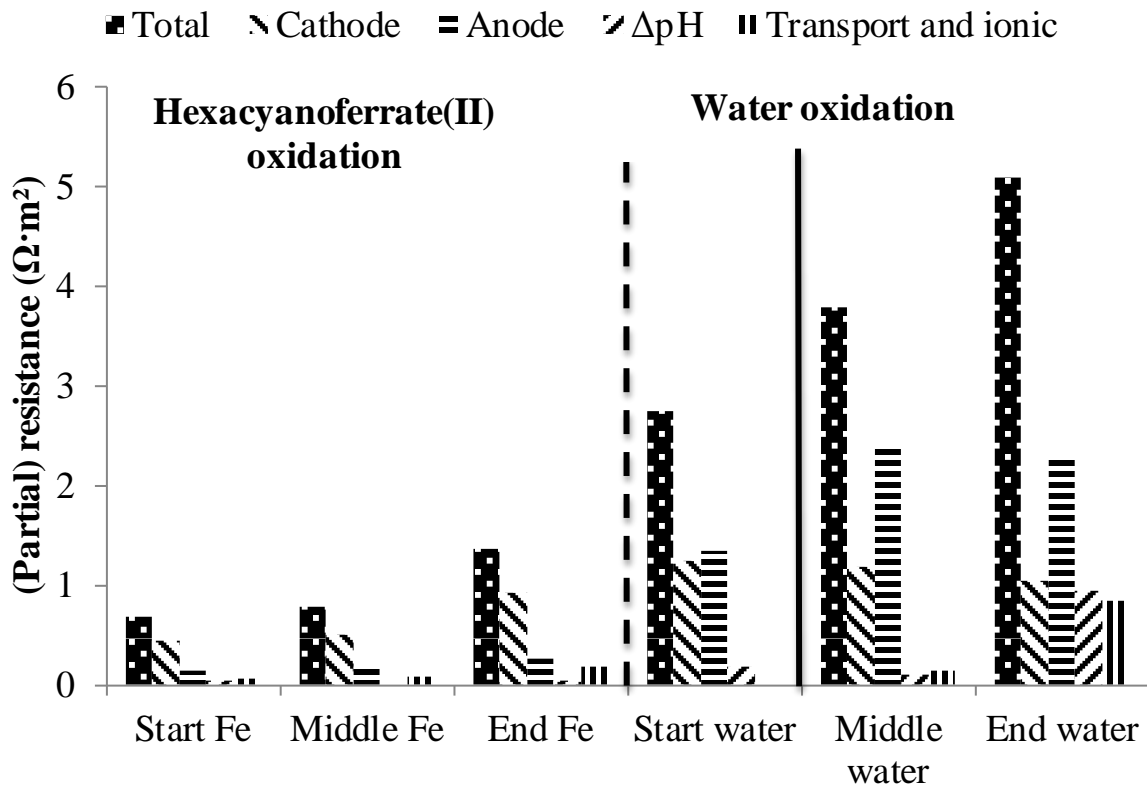
Figure 3. Total internal resistance, and partial resistances were measured in time for hexacyanoferrate(II) and water oxidation at the anode for continuous operation. Anolyte was changed from hexacyanoferrate(II) to water on day 83, as indicated by the dashed line. Anolyte was refreshed on day 115, as indicated by the solid line.

In case of hexacyanoferrate(II) oxidation, the cathode resistance was at least 0.43 $\Omega \cdot m^2$ (start Fe). Immediately after switching anolyte hexacyanoferrate(II) for water, both the cathode and anode contributed most to total internal resistance (Figure 3). Cathode resistance was at least 1.04 $\Omega \cdot m^2$ (end water), while anode resistance was at least 1.33 $\Omega \cdot m^2$ (start water). The sudden increase in anode resistance after switching anolytes could be attributed to the poor catalytic properties of graphite for water oxidation [26]. The increase in anode and cathode resistances with time may be caused by increasing product concentrations (O_2, methane, and protons) near or inside the electrode. This could negatively affect reaction kinetics, and gas accumulation inside the electrode could result in less available effective cathode surface area. Transport & ionic resistances increased with time from 0.015 $\Omega \cdot m^2$ (start water) to 0.83 $\Omega \cdot m^2$ (end water), despite refreshing the anolyte (day 115). In

1 this same time period, anolyte conductivity increased from 0.3 S/m (start water) to 0.7 S/m (end

2 water), while catholyte conductivity decreased from 2.1 S/m (start water) to 1.0 S/m (end water).

3 This is equal to a decrease in ionic resistance from 0.01 $\Omega \cdot m^2$ (start water) to 0.006 $\Omega \cdot m^2$ (end

4 water), using the equation described by Sleutels *et al.* [18] and assuming the distance between the

5 electrodes and the membrane is 5 mm. These calculations show that the transport & ionic resistance

6 mainly consists of transport losses, and not so much of ionic losses due to limited conductivity of the

7 electrolyte, which is in line with Sleutels *et al.* [18]. The resistance due to the development of a pH-

8 gradient over the membrane increased in the same time period from 0.17 $\Omega \cdot m^2$ (start water) to 0.94

9 $\Omega \cdot m^2$ (end water), with the most prominent increase at the end using water oxidation, as the anolyte

10 acidified due to proton production from pH 6.25 (start water; cathode pH 7.09) to 3.37 (end water;

11 cathode pH 7.10). At the end using water oxidation, all resistances contributed to a similar extent to

12 the total internal resistance.

13

14 *Increased methane yield per hectare of land area*

15 At this point, the methane producing MEC is still in its early stage of development. For an

16 estimation of the potential of a methane producing MEC, two crucial inputs need to be considered:

17 renewable electrical energy and CO_2. There are several possible sources and technologies for

18 renewable electricity production: PV cells using solar energy, wind turbines using wind energy, or

19 reverse electrodialysis using the energy from mixing salt and fresh water [27]. A suitable source of

20 CO_2 should (i) be of renewable origin to be independent of fossil fuels, and (ii) contain high

21 concentrations of CO_2, preferably without oxygen present. Gas streams of fermentation processes

22 (renewable fuel production technologies) are therefore an attractive CO_2 source for methane

23 producing MECs. As an example, we will discuss biogas produced via anaerobic digestion of

24 biomass. Biogas consists of both CH_4 and CO_2, which are produced in a 1:1 ratio. To add a higher

25 energetic and economic value to the biogas, it needs to be upgraded, which means that the CO_2

26 content needs to be lowered, and the methane content needs to be increased. Conventionally, CO_2 is

27 removed by scrubbing the CO_2-rich gas with an aqueous solution containing chemicals (hydroxide,

1 amines, etc.) [28]. By contrast, a methane producing MEC does not only lower the CO_2 content of

2 biogas, but furthermore, converts CO_2 into additional methane. As roughly half of the biogas

3 consists of CO_2, the methane yield from anaerobic digestion could be doubled using a methane

4 producing MEC.

5
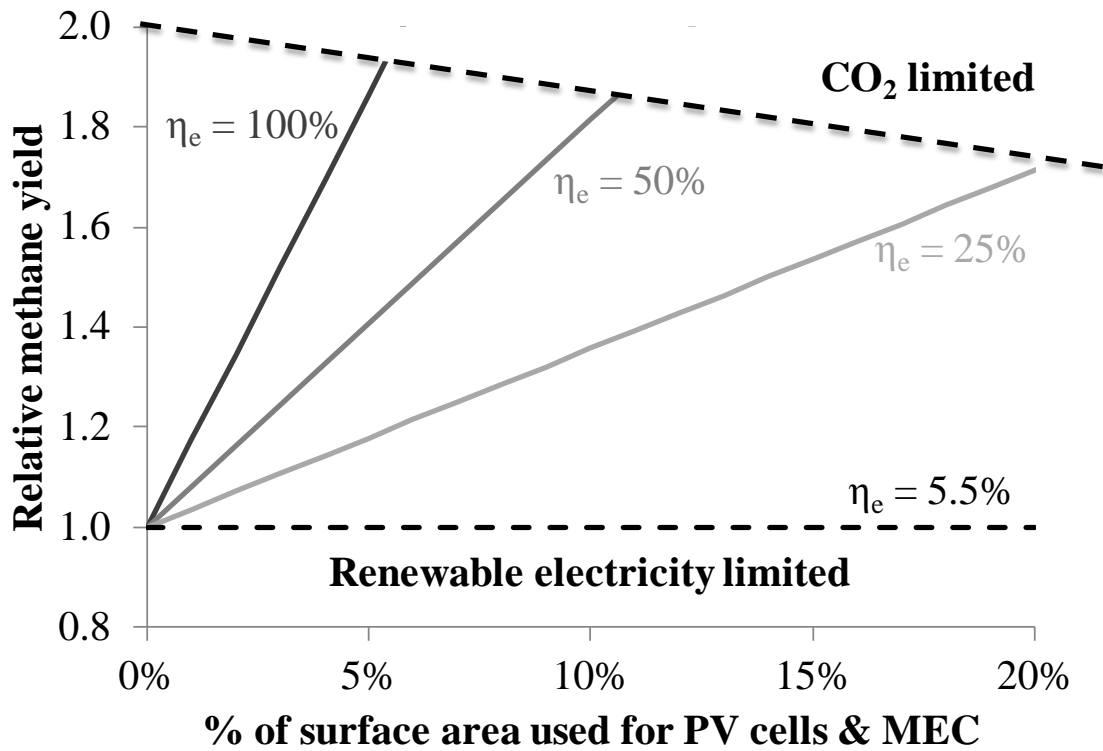
6

7 **Figure 4.** Combining anaerobic digestion and methane producing MECs leads to an improved

8 methane yield per hectare of land area. The energy efficiency (η_e) of the methane producing MEC is

9 calculated as the ratio between the thermodynamic energy input based on the Gibb's free energy of

10 the reaction (9.8 kWh/m^3 methane) divided by the actual electrical energy input. A relative methane

11 yield >1 indicates that more methane is produced than via anaerobic digestion alone. Thus, at an

12 energy efficiency above 5.5%, the combination of anaerobic digestion and methane producing MECs

13 leads to an increased methane yield per hectare of land per year compared to anaerobic digestion.

14

15 Figure 4 shows the relative methane yield per hectare of land area per year for combined anaerobic

16 digestion and methane producing MECs using electricity from PV cells. A relative methane yield >1

17 indicates that more methane is produced via the combined processes compared to anaerobic

18 digestion alone. The relative methane yield is shown as a function of the surface area used for PV

22

cells in combination with a methane producing MEC, and the energy efficiency of a methane producing MEC. We assume that PV cells convert the incoming solar radiation of 150 W/m^2 [29] into electricity at an efficiency of 10% [30], and that biogas consists of equal parts of methane and CO_2. In the hypothetical situation of 100% energy efficiency, which means that no energy losses occur in the MEC system and the energy input consists only of the reversible thermodynamic energy input, to double the methane yield, 5% of the land area needs to be covered with PV cells in combination with methane producing MECs (meaning that 95% of the surface area is used for biomass growth, resulting in a lower CO_2 yield per hectare, and a relative methane yield <2). The methane producing MEC should have an energy efficiency higher than 5.5% to increase methane yield per hectare of land area compared to anaerobic digestion alone (Figure 4).

It is important to note that the use of water oxidation at the anode is essential for reaching an additional methane yield compared to existing biomass conversion technologies. Methane producing MECs that use biomass in the form of acetate as the electron donor at the anode, and that recycle the produced CO_2 from the anode to the cathode, produce the same mix of CO_2 and methane as anaerobic digestion. Oxidation of one mole of acetate at the anode results in 8 moles of electrons and 2 moles of CO_2, the latter being present in the form of bicarbonate at biologically relevant pH 7 (eq. 16). The formed 8 moles of electrons can be used to reduce only one mole of CO_2 (or bicarbonate) to methane (eq. 17). The overall reaction in a methane producing MEC using acetate oxidation at the anode is thus limited by the 8 moles of electrons present in one mole of acetate. Overall, 1 mole of acetate results in 1 mole of methane produced at the cathode, and 1 mole of CO_2 produced at the anode (eq. 18).

Organic matter oxidation at anode [13]:

$$CH_3COO^- + 4H_2O \rightarrow 2HCO_3^- + 9H^+ + 8e^- \tag{16}$$

Carbon dioxide reduction at cathode:

$$HCO_3^- + 9H^+ + 8e^- \rightarrow CH_4 + 3H_2O \tag{17}$$

Overall reaction methane producing MEC using organic matter oxidation at the anode:

1      $CH_3COO^- + H_2O \rightarrow CH_4 + HCO_3^-$ (18)

2

3   In contrast, when using water oxidation at the anode, the amount of electrons is in principle

4   unlimited, meaning that sufficient electrons can be produced to reduce all the CO_2 present into

5   methane, leading to a theoretically double methane yield. Methane producing MECs that use an

6   electron source other than biomass are therefore the only way to achieve higher methane yields per

7   hectare of land area compared to anaerobic digestion.

8

9   *Perspectives*

10   This study showed that an MEC is suitable to convert CO_2 to methane, with a biocathode that

11   continuously converted CO_2 to methane for 188 days. The maximum achieved energy efficiency in

12   this study was 51.3%, obtained during the yield test using water oxidation. An energy efficiency of

13   51.3% would increase the methane yield per hectare of land area by a factor 1.8 when covering 10%

14   of the land area with PV cells (Figure 4). For these calculations, it is assumed that the methane

15   production rate of methane producing MECs is the same as the CO_2 production rate of anaerobic

16   digestion (5 m^3/m^3 per day), whereas the maximum achieved methane production rate in this study

17   was still a factor 25 lower. A considerable reduction in internal resistance is still needed to reach

18   sufficiently high conversion rates. The internal resistance analysis shows that several improvements

19   can be made to reduce the internal resistance and to increase energy efficiency. First, high-surface

20   electrode materials with good catalytic properties for water oxidation and with good properties for

21   biofilm development [19] for catalysis of CO_2 reduction should be used to decrease cathode and

22   anode losses [13]. Second, by directing the flow through the porous electrode, the surface area can

23   be effectively used and mass transfer losses are decreased [13, 31]. Third, decreasing the distances

24   between membrane and electrodes minimizes mass transfer losses even further [13, 32]. Finally, to

25   increase the coulombic efficiency and the methane content of the gas, a membrane that is less

26   permeable for gases should be used. Implementing these improvements will bring a methane

producing MEC closer to its potential for increasing the methane yield land use efficiency, and consequently to increase water, and nutrient efficiency.

ACKNOWLEDGMENT.

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1

Table 1. Performance of a methane producing biocathode with hexacyanoferrate(II) or water oxidation at the anode during continuous operation and for yield tests.

	Hexacyanoferrate(II) oxidation			Water oxidation		
	Continuous		*Yield test*	*Continuous*		*Yield test*
Operation period	Start Fe	Middle Fe	Day 70	Middle water	End water	Day 101
E_{cat} *(V)*	-0.69	-0.71	-0.69	-0.55	-0.55	-0.70
j *(A/m^2)*	0.87	0.80	1.09	0.25	0.21	1.75
r_{CH4} *(m^3/m^3 per day)*	0.071	0.033	0.15	0.005	0.006	0.21
$\eta_{coulombic}$ *(%)*	64.9	33.2	104.6	17.5	23.1	95.2
Energy input (kWh/m^3)	15.4	31.6	16.6	97.2	73.5	18.2
$\eta_{voltage}$ *(%)*	63.7	31.1	59.3	10.1	13.4	53.9
η_{energy} *(%)*	42.6	10.3	62.0	1.8	3.1	51.3

2