Biocatalyzed hydrogen production in a continuous flow microbial fuel cell with a gas phase cathode

B. Tartakovsky [a,∗], M.-F. Manuel [a], V. Neburchilov [b], H. Wang [b], S.R. Guiot [a]

[a] *Biotechnology Research Institute, National Research Council of Canada, 6100 Royalmount Avenue, Montreal, QC, Canada H2P 2R2*
[b] *Institute for Fuel Cell Innovation, National Research Council of Canada, 4250 Wesbrook Mall, Vancouver, BC, Canada V6T 1W5*

A B S T R A C T

A single liquid chamber microbial fuel cell (MFC) with a gas-collection compartment was continuously operated under electrically assisted conditions for hydrogen production. Graphite felt was used for anode construction, while the cathode was made of Pd/Pt coated Toray carbon fiber paper with a catalyst loading of $0.5\,mg\,cm^{-2}$. To achieve hydrogen production, the MFC was connected to a power supply and operated at voltages in a range of 0.5–1.3 V. Either acetate or glucose was used as a source of carbon. At an acetate load of $1.67\,g\,(L_A\,d)^{-1}$, the volumetric rate of hydrogen production reached $0.98\,L_{STP}\,(L_A\,d)^{-1}$ when a voltage of 1.16 V was applied. This corresponded to a hydrogen yield of $2\,mol\,(mol\text{-}acetate)^{-1}$ with a 50% conversion efficiency. Throughout the experiment, MFC efficiency was adversely affected by the metabolic activity of methanogenic microorganisms, which competed with exoelectrogenic microorganisms for the carbon source and consumed part of the hydrogen produced at the cathode.

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1. Introduction

Growing energy needs and rising concern about fossil fuel emissions and climate change have directed research towards alternative fuels and energy producing technologies. One such technology is energy production in a microbial fuel cell (MFC). Although most MFC studies have focused on electricity production [1–7], the application of MFCs for hydrogen production has been recently demonstrated [8–10].

In an electrically assisted MFC, organic materials are converted into hydrogen rather than electricity. Here, microbial degradation of organic matter at the anode results in a release of electrons and protons. Electrons are transferred from the anode to the cathode through an external circuit. Hydrogen formation at the cathode from the protons requires additional energy input, which is provided by a power supply [10,11]. Because organic matter rather than water is the source of protons for hydrogen formation, this process can be referred to as biocatalyzed electrolysis [9,10]. As well, the term exoelectrogenic hydrogen production [12] can be used to highlight extracellular transfer of electrons to the anode.

In theory, a voltage of 0.14–0.22 V is sufficient for hydrogen production in a MFC. However, because of electrode overpotentials and

other losses, hydrogen production is observed starting from 0.25 to 0.5 V [11]. Nevertheless, this is still below the theoretical threshold of 1.23 V and well below the practical threshold of 1.8 V required for water electrolysis [10]. Thus, hydrogen production in a MFC can be achieved at significantly lower power consumption when compared to hydrogen production by water electrolysis. Because this process can utilize a diverse range of organic materials, hydrogen production can be combined with wastewater treatment or organic waste conversion.

The main limitations associated with hydrogen production in an electrically assisted MFC include low volumetric efficiency and the use of an expensive catalyst (platinum). This study attempts to address these issues by using a single liquid chamber MFC with a three-dimensional carbon felt anode and a Pd/Pt cathode.

2. Materials and methods

2.1. Media composition

The stock solution of carbon source was composed of (in $g\,L^{-1}$): either glucose (40.0) or acetate (40.0) in a solution of yeast extract (6.7), NH_4Cl (18.7), KCl (148.1), K_2HPO_4 (64.0), and KH_2PO_4 (40.7). The stock solution of the trace metals was prepared according to [9] and contained (in $mg\,L^{-1}$): $FeCl_2\cdot4H_2O$ (2000), H_3BO_3 (50), $ZnCl_2$ (50), $CuCl_2$ (30), $MnCl_2\cdot4H_2O$ (500), $(NH_4)_6Mo_7O_{24}\cdot4H_2O$ (50), $AlCl_3$ (50), $CoCl_2\cdot6H_2O$ (50), $NiCl_2$ (50), EDTA (500), and HCl (1 mL). All solutions were filter sterilized and maintained at 4 °C

∗ Corresponding author. Tel.: +1 514 496 2664; fax: +1 514 496 6265.
E-mail address: Boris.Tartakovsky@nrc-cnrc.gc.ca (B. Tartakovsky).

until use. Distilled water was used for solution preparation, and the chemicals and reagents used were of analytical grade.

2.2. Analytical measurements

Volatile fatty acids (VFAs) were analyzed on an Agilent 6890 gas chromatograph (Wilmington, DE) equipped with a flame ionization detector and a 1 m × 2 mm 60/80 mesh Carbopack C column (Supelco, Bellafonte, PA, USA) coated with 0.3% Carbowax 20 M and 0.1% H_3PO_4. The carrier gas was helium, which had a flow rate of 20 mL min^{-1}. The injector and the detector were maintained at 200 °C. A 0.5 μL samples were fortified at a ratio of 1:1 (v/v) using an internal standard of *iso*-butyric acid dissolved in 6% formic acid. Glucose was analyzed on an HPLC (Waters Chromatography, Milford, MA, USA) equipped with a PDA detector model 2996.

Gas production in MFC was measured on-line by means of bubble counters connected to glass U-tubes and interfaced with a data acquisition system. The U-tubes contained a dye, which facilitated bubble counting. The gas composition was measured using a gas chromatograph (6890 Series, Hewlett Packard, Wilmington, DE) equipped with a 11 m × 3.2 mm 60/80 mesh Chromosorb 102 column (Supelco, Bellefonte, PA, USA) and a flame ionization detector. Carrier gas was argon.

2.3. Cathode fabrication

The cathode was fabricated using a Toray carbon fiber paper impregnated with 10% PTFE (ETEK Inc., USA) and a carbon supported Pd/Pt alloy catalyst. The carbon supported Pd/Pt alloy catalyst (50%Pt–50%Pd)/C, was obtained from solutions of $PdCl_2$ and H_2PtCl_6 following the borohydride method described by Raghuveer et al. [13].

Before the catalyst layer was applied onto the carbon fiber paper, a microporous carbon sublayer (carbon loading is 1 mg cm^{-2}) was coated on the carbon fiber paper first by using an automated sprayer (Ultra TT Series, EFD A Nordson Co., USA) with a 781S Series spray valve SS-WF and a round air cap nozzle. A catalyst ink was prepared by mixing the carbon supported Pd/Pt alloy catalyst powder (40% metal on carbon), 5% Nafion suspension and *iso*-propanol/water (1:1) mixture with the help of a sonicator at a temperature of 80 °C. The catalyst ink was then applied on top of the carbon sublayer by the same automated sprayer. The electrode was finally dried in a vacuum oven at 100 °C for 3 h to remove the solvent. The catalyst loading was 0.5 mg Metal cm^{-2}. A Nafion 117 membrane was hot-pressed onto the cathode to form the partial membrane electrode assembly (MEA). A second partial MEA was fabricated by hot-pressing a Nafion 117 membrane onto an E-TEK gas diffusion electrode (GDE) with a Pt load of 0.5 mg cm^{-2} (GDE LT 120EW, E-TEK Division, PEMEAS Fuel Cell Technologies, Somerset, NJ, USA).

2.4. MFC design, instrumentation, and operation

All experimentation was carried out in continuously operated MFCs. Two cells were constructed, each with a series of polycarbonate plates arranged to form two chambers. The anodic chamber retained 60 mL of liquid and had a headspace of 40 mL. The gas-collection chamber had a volume of 50 mL. A 1.6 mm thick Neoprene gaskets provided gas tight connections between the plates. The cells were equipped with lines for influent, effluent, liquid recirculation and gas exits (Fig. 1). Gas tightness was provided by glass U-tubes installed on all exit lines. The U-tubes installed on gas lines were equipped with bubble counters, as described above.

The liquid filled (anodic) chamber housed the anode, which was made of a 5 mm thick graphite felt measuring 10 cm × 5 cm (Speer Canada, Kitchener, ON, Canada). The partial MEA was secured

between two plates. In the MFC with Pd/Pt cathode, the distance between the anode and cathode was 20 mm, while in the MFC with Pt GDE cathode a distance of 3–5 mm was used. The second chamber of each MFC contained no liquid and was used for gas collection (Fig. 1). The MFCs were inoculated with 5 mL of homogenized anaerobic sludge (Rougemont, QC, Canada).

A stock solution of carbon source was fed using an infusion pump (model PHD 2000, Harvard Apparatus, Canada) at a rate of 2.5–5 mL d^{-1}. One millilitre of trace metals stock solution was added to 1 L of the dilution water. The dilution water was fed at a rate of 146 mL d^{-1} using a peristaltic pump (Cole-Parmer, Chicago, IL, USA) providing a retention time of 10 h. Homogeneous distribution of the carbon source in the anodic chamber was provided by an external recirculation loop. A recirculation rate of 0.57 L h^{-1} was used in all experiments.

MFC temperature was maintained at 25 °C by means of a thermocouple placed in the anodic chamber, a temperature controller (Model JCR-33A, Shinko Technos Co., Ltd., Osaka, Japan) and a 5 cm × 10 cm heating plate located on the anodic chamber side of the MFC. pH was maintained at a set-point of 7.0 using a pH probe installed in the recirculation line, a pH controller (Model PHCN-410, Omega Engineering, Stamford CT, USA) and a solution of 0.05N NaOH, which was fed to the recirculation line. During electricity-production mode, MFCs were routinely operated at an external resistance of 400 Ω and the gas-collection chambers were exposed to atmosphere by opening gas lines located at the top and the bottom of the chamber (Fig. 1). Voltage was measured on-line at 10 min intervals using a data acquisition system (Labjack U12, Labjack Corp., Lakewood, CO, USA). In electrically assisted mode, a 15 Ω resistor was added to the circuit for current measurements, which were also conducted at 10 min intervals. To account for power losses at the resistor, applied voltage was measured directly at the MFC. An adjustable DC power supply (IF40GU Kenwood, Japan) was used to maintain voltage at the preset setpoint.

3. Results

3.1. Startup procedures

Since the startup procedure was identical for both MFCs, startup of MFC with Pd/Pt cathode is described below. Operation was started up in electricity-production mode. Glucose, fed to anodic chamber at a load of 3.33 g $(L_A d)^{-1}$ (A = anodic chamber), was used as a sole source of carbon. To follow the process of anode colonization by exoelectrogenic microorganisms, a 400 Ω external resistance was connected to the electrodes so that voltage evolution in time could be monitored. Voltage began to increase after 10 days of MFC operation and within the following 3 days had stabilized at 420–430 mV, corresponding to a volumetric power production of 7.0 mW L_A^{-1}. A polarization test was conducted on Day 15 by varying the external resistance from 25 to 1000 Ω with 1 min intervals between the measurements. A maximum volumetric power production of 13.7 mW L_A^{-1} was recorded in this test (Fig. 2A). Based on the linear part of the current–voltage curve, internal cell resistance was estimated at 67 Ω. Notably, at external resistances below the cell's internal resistance a significant decrease in the current generated by the MFC was observed. Apparently, in this domain power production was limited by several factors, particularly metabolic activity of exoelectrogenic microorganisms and the rate of electron transfer to the anode by the microorganisms. Nevertheless, MFC performance was considered satisfactory to proceed to the hydrogen production test.

Hydrogen production was initiated by flushing the gas-collection chamber with nitrogen and connecting the anode and
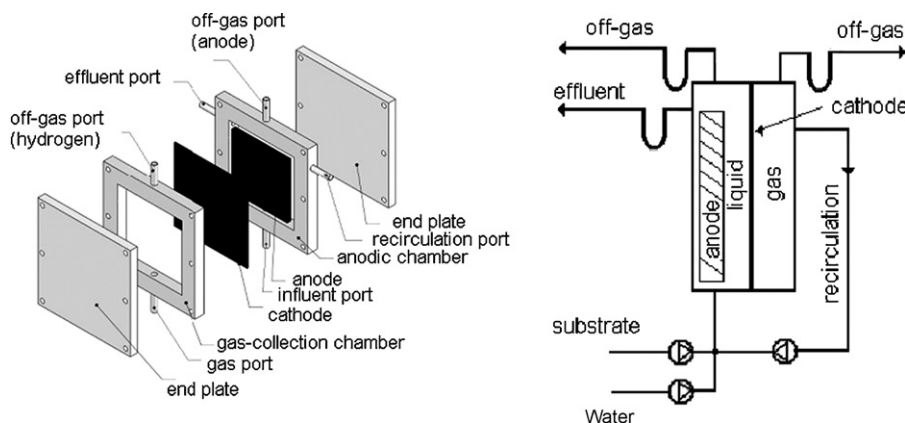
Fig. 1. MFC schematic (left) and continuous flow MFC setup (right). Carbon source and nutrients were continuously fed through the influent line using an infusion pump and a peristaltic pump, respectively.
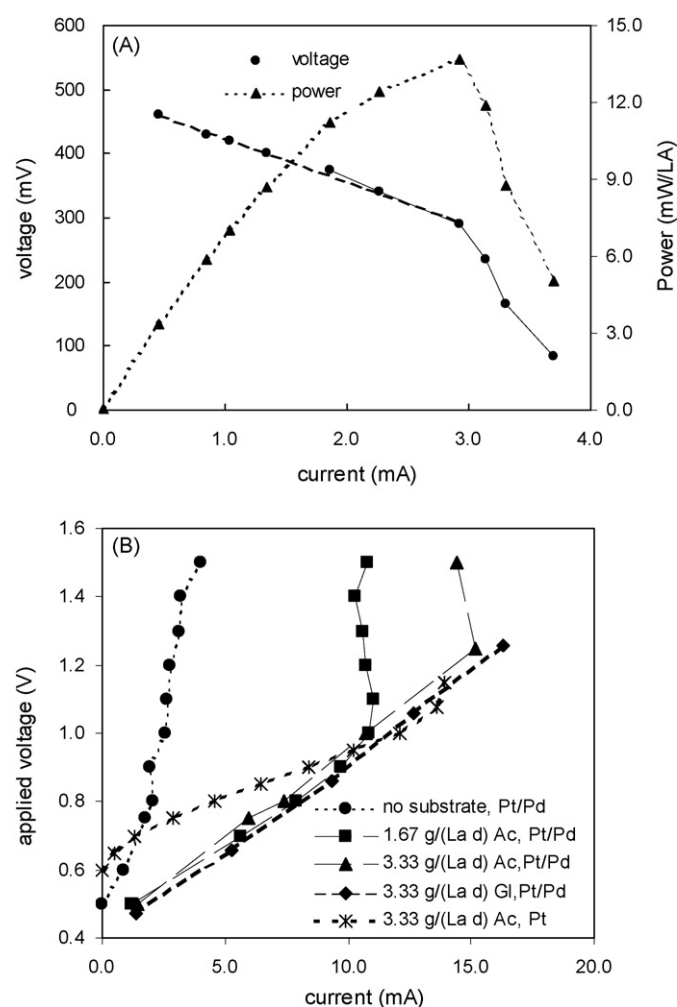

Fig. 2. Polarization curve obtained in electricity-production mode (A) and voltage scans obtained in hydrogen-production mode using either glucose or acetate with Pd/Pt cathode and acetate with Pt cathode (B).

cathode terminals to positive and negative terminals of a power supply, respectively. A voltage of 0.7 V was applied to the MFC. Also, the carbon source was changed from glucose to acetate and organic load was decreased to $1.67\,g\,(L_A\,d)^{-1}$ ($680\,mg\,L^{-1}$ of acetate in the influent stream). Hydrogen production started almost immediately. At steady state gas flow from the gas-collection chamber

was $0.66 \pm 0.02\,L_{STP}\,(L_A\,d)^{-1}$ and this gas contained 70–75% H_2, 12–16% CH_4, 5–10%N_2, and 3% water vapor. No carbon dioxide was detected. Gas production was also observed in the anodic chamber with an average flow rate of $0.21 \pm 0.04\,L_{STP}\,(L_A\,d)^{-1}$. The anodic chamber off-gas on average contained no H_2, 60–65% CH_4, 5–6% CO_2, 30–35% N_2 and water vapor.

3.2. Hydrogen production from acetate

For the MFC with Pd/Pt cathode, the startup acetate load of $1.67\,g\,(L_A\,d)^{-1}$ was doubled to $3.3\,g\,L_A^{-1}\,L_A\,d^{-1}$, while maintaining voltage at 0.7 V. A 7-day period was allowed to ensure a new steady state. The increase in acetate load did not result in a significant change in hydrogen production. Gas flow rate in the gas-collection chamber remained at $0.66\,L_{STP}\,(L_A\,d)^{-1}$ and gas composition was unchanged. Gas production in the anodic chamber, however, increased to $0.49\,L_{STP}\,(L_A\,d)^{-1}$ with a CH_4 content of 65–68%, i.e. methane production rate in the anodic chamber became comparable to the rate of hydrogen production. Effluent acetate concentration increased and stabilized at $640–680\,mg\,L^{-1}$. Consequently, the acetate load was restored to $1.67\,g\,(L_A\,d)^{-1}$ and this load was used in subsequent tests.

To estimate hydrogen production at different applied voltages, a range of voltages between 0.5 and 1.3 V was used and the MFC was operated for at least 2 days (5 HRTs) at each voltage. The applied voltages were randomly selected to minimize the effect of microbial adaptation on the hydrogen production rate. At a voltage of 0.5 V a rapid drop in hydrogen concentration with a simultaneous increase in methane concentration was observed. Furthermore, pressure in the gas-collection chamber became negative, as was evidenced by reversed gas flow in the U-tube installed at the gas exit (Fig. 1). Resuming a voltage of 0.7 V restored the hydrogen production rate and gas composition to previous values. Further voltage increase resulted in a proportional increase in hydrogen production, which reached $0.98\,L_{STP}\,(L_A\,d)^{-1}$ at a voltage of 1.16 V (Fig. 3A). As voltage increased, effluent concentration of acetate gradually decreased from 190 to $68\,mg\,L^{-1}$. However, voltage increase to 1.3 V resulted in a decreased rate of hydrogen production, while effluent acetate concentration remained unchanged (Fig. 3A). A summary of measured gas flow rates and acetate removal rates at each voltage is given in Table 1.

For MFC with Pt cathode, similar COD removal rates were observed ($1.5–1.6\,g\,(L_A\,d)^{-1}$ at 1.0–1.1 V). At an applied voltage of 1.0 V, the volumetric rate of hydrogen production was at $0.96\,L_{STP}\,(L_A\,d)^{-1}$, which was similar to the rate observed when using Pd/Pt loaded cathode.

Table 1
Measurements of substrate removal and gas production during tests of hydrogen production from acetate

Voltage (V)	COD removal $(g(L_A d)^{-1})$	Anodic CH_4 $(L_{STP}(L_A d)^{-1})$	Cathodic CH_4 $(L_{STP}(L_A d)^{-1})$	Cathodic H_2 $(L_{STP}(L_A d)^{-1})$	Calculated COD recovery (%)
0.7	1.28	0.16	0.14	0.48	98
0.96	1.48	0.12	0.11	0.85	89
1.16	1.57	0.12	0.15	0.98	96
1.26	1.58	0.12	0.15	0.82	88

The tests were carried out at an acetate load of $1.67\,g(L_A d)^{-1}$. Standard deviation was estimated at 0.04 and $0.14\,L_{STP}(L_A d)^{-1}$ for CH_4 and H_2 measurements, respectively. COD recovery was calculated using yields of $Y_{CH_4} = 0.35\,L_{STP}\,g^{-1}$ and $Y_{H_2} = 1.49\,L_{STP}\,g^{-1}$ for methane and hydrogen, respectively.

3.3. Hydrogen production from glucose

Hydrogen production when using glucose as a source of carbon was tested in the MFC with a Pd/Pt cathode by replacing acetate in the stock solution with an equivalent amount of glucose on a COD basis. At a glucose load of $1.67\,g(L_A d)^{-1}$ sampling of the anodic chamber showed trace amounts of acetate and propionate ($15–20\,mg\,L^{-1}$ each) with no detectable amounts of glucose, even though influent glucose concentration was $680\,mg\,L^{-1}$. At this load and a voltage of 0.7 V, a hydrogen production rate of $0.34\,L_{STP}(L_A d)^{-1}$ was measured. Methane production rate in the anodic chamber was below $0.05\,L_{STP}(L_A d)^{-1}$. When glucose load

Fig. 3. Hydrogen production rate and effluent acetate concentrations at different voltages in a MFC fed with acetate at a load of $1.67\,g(L_A d)^{-1}$ (A) and fed with glucose at a load of $3.33\,g(L_A d)^{-1}$ (B).

was increased to $3.33\,g(L_A d)^{-1}$, hydrogen production remained unchanged while anodic off-gas flow increased to $0.23\,L_{STP}(L_A d)^{-1}$. The anodic gas mainly consisted of CH_4 (60%) and N_2 (30%). Also, effluent concentrations of acetate and propionate increased to $60\,mg\,L^{-1}$ and $250\,mg\,L^{-1}$, respectively. Because of low effluent concentrations observed at a load of $1.67\,g(L_A d)^{-1}$, hydrogen production measurements were conducted at the increased load. As in the acetate tests, voltage increase resulted in improved volumetric efficiency of hydrogen production and reduced concentration of VFAs in the effluent (Fig. 3B). At a voltage of 1.2 V hydrogen production increased to $0.58\,L_{STP}(L_A d)^{-1}$ and effluent VFA concentration declined from 370 to $240\,mg\,L^{-1}$. However, further increase in voltage to 1.5 V led to a sharp decrease in both hydrogen production and COD removal efficiency.

3.4. Voltage scans

Throught the experimental period, several voltage scans were conducted by varying the voltage from 0.5 to 1.5 V with 5 min intervals between each voltage change (Fig. 2B). In the MFC with Pd/Pt cathode, a scan obtained at an acetate load of $1.67\,g(L_A d)^{-1}$ showed near linear increase of current with increasing voltage up to 1.0 V. This was followed by current stabilization so that the maximal rate of hydrogen production was predicted to be at 1.0–1.1 V. Another scan conducted at an acetate load of $3.33\,g(L_A d)^{-1}$ produced a curve, which was similar to that obtained at $1.67\,L_A^{-1}\,d^{-1}$, except that the current above 1.1 V was higher (Fig. 2B). A scan conducted at a glucose load of $3.33\,g(L_A d)^{-1}$ produced a curve similar to that at an acetate load of $3.33\,g(L_A d)^{-1}$. Based on the linear parts of all curves an internal resistance of $51\,\Omega$ was estimated, which was comparable to $67\,\Omega$ estimated from the polarization test. A voltage scan was also conducted for the MFC with a Pt cathode. At an acetate load of $3.33\,g(L_A d)^{-1}$, which was used to ensure substrate non-limiting conditions, current at applied voltages below 1.0 V was less than that obtained with the Pd/Pt cathode, suggesting higher overpotentials as compared to the Pd/Pt cathode (Fig. 2B, asterisks).

To estimate energy losses due to non-biological reactions, a voltage scan was also conducted in the absence of a carbon source by removing the carbon source from the stock solution while maintaining all other components. The Pd/Pt-MFC was operated without the carbon source (acetate) for a period of 3 days before the scan. The resulting values (Fig. 2B) were approximated by a linear equation, which was subsequently used to correct for background currents. Interestingly, as soon as the acetate feed was stopped, a rapid increase in methane and decrease in hydrogen concentrations was observed in the gas-collection chamber, so that hydrogen declined from 77% to 9%. As was already observed in the tests conducted at 0.5 V, gas flow in the gas-collection chamber was reversed, i.e. the pressure became negative.

4. Discussion

Overall, hydrogen was successfully produced from organic matter at voltages from 0.7 to 1.2 V, i.e. below a theoretical water
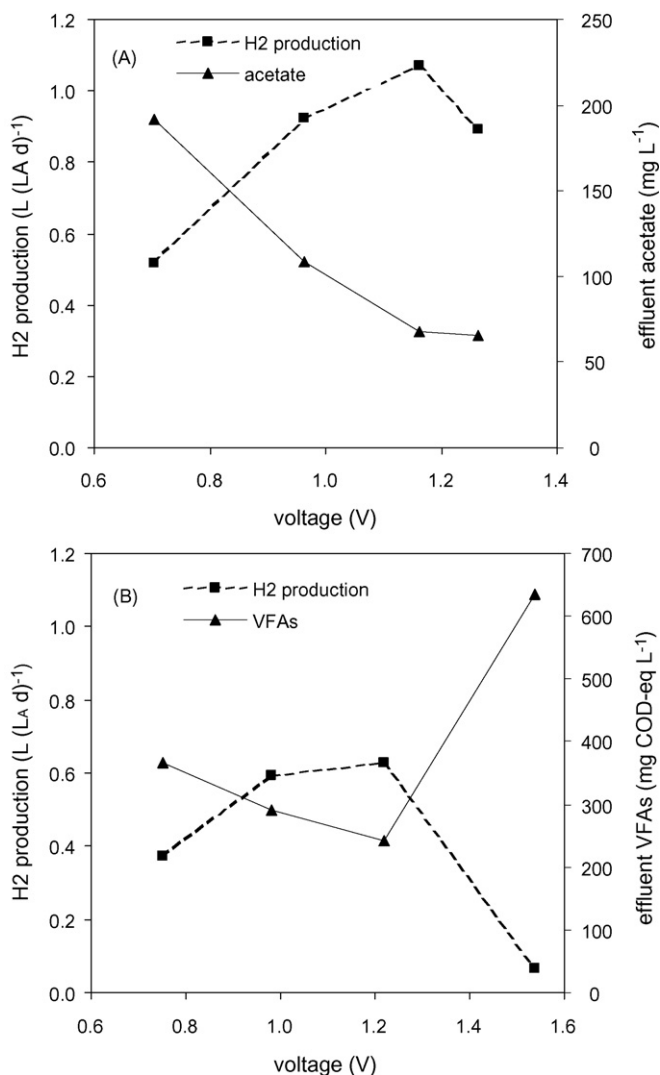
electrolysis threshold of 1.23 V [10]. Importantly, hydrogen formation was observed when acetate was used as a sole carbon source. Thus, limitations of biological hydrogen production in the fermentation process (dark fermentation) were resolved [10,11]. Stoichiometry of glucose to hydrogen transformation suggests that 12 moles of hydrogen can be produced from a mole of glucose if complete oxidation to carbon dioxide can be achieved [14]. However, biochemical limitations result in acetate being the final product of the fermentation, therefore limiting the hydrogen yield to only four moles. In practice, butyrate and other volatile fatty acids are also formed, which further decreases the hydrogen yield from glucose to 2 mol mol^{-1} or below [11].

Interestingly, it appears that VFAs were used by exoelectrogenic microorganisms even when MFC was fed with glucose. Measurements of glucose and VFA concentrations in the anodic chamber of MFC fed with glucose showed the presence of acetate and propionate, while glucose concentration was below the detection limit. The MFC was inoculated with anaerobic sludge containing a mixed anaerobic consortium with significant acidogenic activity. Apparently, a rapid conversion of glucose to VFAs by acidogenic bacteria occurred. Thus, it can be hypothesized that exoelectrogenic microorganisms utilized VFAs rather than glucose. In order to achieve direct electron transfer, exoelectrogens are expected to be in close proximity to the anode surface [15], i.e. inside the biofilm. Therefore, a layered biofilm may be formed, where exoelectrogens are located at the biofilm core and acidogenic microorganisms proliferate at the biofilm surface. Biofilm stratification is often observed in reactors where anaerobic biofilms exist and is beneficial for the biodegradation of complex organic molecules [16].

Volumetric efficiency of hydrogen production was highest at around 1.2 V. Also, this voltage corresponded to the lowest effluent acetate concentration (Fig. 3A). Further voltage increase did not result in increased current (Fig. 2B). It can be suggested that at applied voltages above 1.2 V electron transfer became limited by the metabolic activity of the exoelectrogens. Similar restrictions were observed when operating the MFC in the electricity-production mode at external resistances below the internal resistance value (Fig. 2A). In fact, a voltage increase above 1.2 V resulted in decreased hydrogen production, likely due to trace amounts of oxygen produced at the anode.

Relatively high voltage required for hydrogen production can be attributed to energy losses due to electrode overpotentials as well as to non-biological reactions [9]. Nevertheless, the applied voltage was always below the water electrolysis threshold. Furthermore, material balance calculations provided below strongly suggested that part of the hydrogen produced was converted to methane due to activity of hydrogenotrophic methanogenic microorganisms. Because of this biological reaction, the apparent voltage requirements were increased. Overall, the tests suggested that in order to maximize the volumetric rate of hydrogen production, a MFC should be operated at the highest attainable current while avoiding excessive power losses. Since overpotentials are proportional to applied voltage, an optimal operational point can be obtained by taking into account volumetric efficiency as well as power consumption, i.e. by formulating and solving an optimization problem.

The volumetric rate of hydrogen production in the MFC with Pd/Pt cathode reached 0.98 L$_{STP}$(L$_A$ d)$^{-1}$. This is a significant improvement in comparison to a volumetric rate of 0.0045 L(L$_A$ d)$^{-1}$ reported by Logan and co-workers [11]. The improvement can be attributed both to improved reactor design and the use of carbon felt as the anode material. Carbon felt has been demonstrated to be suitable for colonization by anaerobic microorganisms [17]. Recent studies demonstrated direct electron transfer by exoelectrogenic microorganisms [15], i.e. volumetric efficiency might be improved when using materials with large surface area,

such as carbon felt. Indeed, a volumetric rate of 0.3 L(L$_A$ d)$^{-1}$ was reported by Rozendal et al. [9] for a carbon felt anode.

A calculation based on influent and effluent acetate concentrations suggested a hydrogen yield of 2 mol-H$_2$ (mol-acetate)$^{-1}$ at an applied voltage of 1.16 V. With respect to a maximal theoretical yield of 4 mol mol^{-1} calculated based on complete acetate oxidation to carbon dioxide, this corresponded to a 50% efficiency. However, this estimation did not account for acetate consumption by methanogenic microorganisms, which was significant throughout the experiment. Indeed, methane production in the anodic chamber off-gas contained 60–65% methane. Moreover, up to 16% methane was found in the gas-collection chamber off-gas. Therefore, the existence of a mixed exoelectrogenic–methanogenic microbial community should be accounted for in the MFC material balance. Consequently, a simplified material balance of a continuous flow MFC was written as follows (in g-COD d^{-1}):

$$Q_{in} = Q_{out} + Q_{H_2} + Q_{CH_4}, \tag{1}$$

where Q_{in} and Q_{out} are the influent and effluent substrate fluxes, respectively, Q_{H_2} the flux of substrate used by the exoelectrogenic microorganisms; and Q_{CH_4} is the flux of substrate used by the methanogenic microorganisms. The latter can be estimated using the theoretical methane yield of $Y_{CH_4} = 0.35$ L$_{CH_4}$ g^{-1} COD, which allows for Q_{CH_4} estimations using methane measurements:

$$Q_{CH_4} = \frac{F_{CH_4}}{Y_{CH_4}}, \tag{2}$$

where F_{CH_4} is the methane production rate (L$_{CH_4}$ d^{-1}). Substrate consumption for hydrogen formation can be estimated by using either current or hydrogen measurements. Notably, current-based measurements are not affected by further hydrogen transformations. Since hydrogen losses to methane formation by hydrogenotrophic methanogens were hypothesized, Q_{H_2} was estimated based on current measurements [11]:

$$Q_{H_2} = \frac{I\Delta t M}{FnC_E}, \tag{3}$$

where I is the current (A), Δt the time period (d), F the Faraday constant (C mol^{-1}), n the number of electrons ($n = 8$ for acetate), M the substrate molecular mass (g), and C_E is the Coulombic efficiency of proton reduction at the cathode (dimensionless). As in methane yield calculations (Eq. (2)), substrate consumption for biomass growth and maintenance was not considered, i.e. $C_E = 1$.

When substrate equivalents were calculated using methane production measurements in the anodic and gas-collection chambers (Eq. (2)) and current measurements (Eq. (3)), the total amount of recovered substrate exceeded the amount fed to the MFC by 15–20% (Fig. 4A and B). The excess corresponded to the amount of methane found in the gas-collection chamber. Initially it was thought that this methane was also produced in the anodic chamber by acetoclastic methanogenic microorganisms and diffused to the gas-collection chamber through the cathode assembly. However, as discussed below, several observations suggested that, in fact, this methane was formed by hydrogenotrophic methanogenic microorganisms from hydrogen produced at the cathode and, therefore, was already accounted for in Q_{H_2} calculations, which were based on current measurements (Eq. (3)). By excluding methane found in the gas-collection chamber from the material balance, a substrate recovery of 85–92% was obtained, as shown in Fig. 4B.

Similar accuracy was obtained when material balance was calculated using process stoichiometry rather than current measurements. In this case a hydrogen yield of 4 mol of H$_2$ per mol of acetate ($Y_{H_2} = 1.49$ L$_{H_2}$ g^{-1}) was assumed and Q_{H_2} was calculated according to $Q_{H_2} = F_{H_2}/Y_{H_2}$, where F_{H_2} is the hydrogen flow
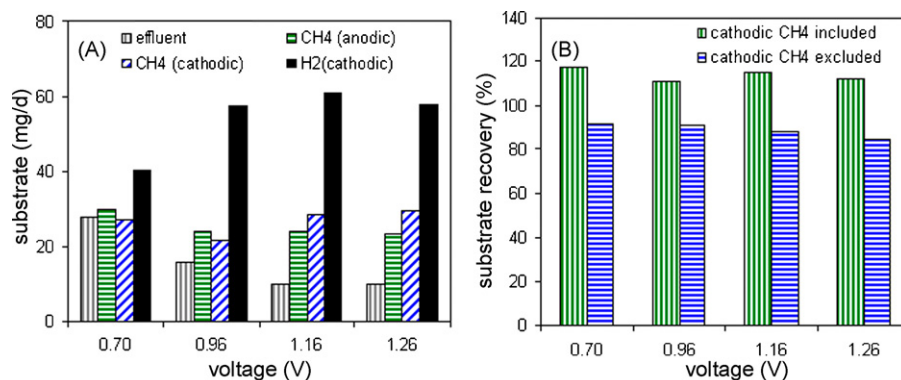
Fig. 4. Material balance calculations showing estimations of substrate consumption for hydrogen and methane production (A) and substrate recovery expressed as % of influent substrate calculated including and excluding methane found in the gas-collection (cathodic) chamber (B). Part of substrate required for hydrogen production was estimated using current measurements and assuming a Coulombic efficiency of $C_E = 1$. Part of substrate required for methane production was calculated assuming a methane yield of $Y_{CH_4} = 0.35 \, L \, g^{-1}$.

rate. Both anodic and cathodic methane production was considered according to Eq. (2). This calculation showed a substrate recovery of 88–98% (Table 1).

These material balance calculations clearly indicated that both acetoclastic and hydrogenotrophic methanogenic microorganisms were present. At the highest hydrogen production rate corresponding to a voltage of 1.16 V only 55–65% of acetate was used for hydrogen production, while up to 30% was used for methane formation in the anodic chamber by acetoclastic methanogenic microorganisms. Furthermore, up to half of the hydrogen produced at the cathode was lost to the activity of hydrogenotrophic methanogens. This explains the relatively high voltages required to observe hydrogen production and implies that the volumetric production rate of hydrogen at a voltage of 1.16 V was close to $2 \, L_{H_2} \, (L_A \, d)^{-1}$, although only $0.98 \, L_{H_2} \, (L_A \, d)^{-1}$, was experimentally observed.

Another confirmation of the existence of mixed methanogenic–exoelectrogenic microbial community in the MFC came from the observed hydrogen disappearance and appearance of methane in the gas-collection chamber in the absence of acetate. A similar trend was observed at a voltage of 0.5 V, i.e. when a low rate of hydrogen production was expected. In either case not only did hydrogen concentration decline and methane concentration increase, but also the chamber pressure became negative. Notably, methane formation from hydrogen requires one mole of carbon dioxide and four moles of hydrogen for each mole of methane according to the following stoichiometric equation:

$$4H_2 + CO_2 \rightarrow CH_4 + H_2O \tag{4}$$

i.e. this reaction might be accompanied by a reduction in gas volume and carbon dioxide consumption. Indeed, CO_2 was never detected in the gas-collection chamber while the anodic chamber on average contained 5.5% CO_2 and 60–65% CH_4. Anaerobic digestor off-gas typically contains 50–70% methane and 30–50% CO_2 at neutral pH, i.e. CO_2 concentration in the anodic chamber off-gas was lower than expected. Likely, at a voltage of 0.5 V the rate of hydrogen consumption by hydrogenotrophic methanogens exceeded the rate of hydrogen production at the cathode.

Visual inspection of the partial MEA at the end of the experiment revealed that the PEM had become partially detached from the cathode and liquid accumulated between the PEM and the surface of the cathode. The presence of biofilm was observed on the cathode as well as on the PEM. Close proximity of hydrogenotrophic methanogens to the cathode facilitated the biotransformation as it provided an alternative hydrogen sink and this reaction proceeded at a significant rate as hydrogenotrophic methanogens feature higher biotransformation rates.

Based on the conclusions of the material balance analysis, methane found in the gas-collection chamber was converted to hydrogen equivalent and the resulting values were used to re-calculate power requirements. The re-calculated values agreed well with the previously published results [9,12] showing a consumption of 1.7–$2 \, W \, (L_{H_2})^{-1}$ at 0.7–1.16 V. Also, while an apparent Coulombic efficiency of 67% was obtained at a voltage of 1.16 V based on hydrogen measurements, a recalculation with respect to hydrogen losses to methane formation yielded a 89% efficiency.

A comparison of Pt and Pd/Pt cathodes demonstrated similar hydrogen production and COD removal efficiencies. However, the Pd/Pt cathode featured lower overpotentials at applied voltages below 1.0 V (Fig. 2B). This was reflected in power consumption, which was estimated at 1.7–$2 \, W \, (L_{H_2})^{-1}$ and 3.2–$3.4 \, W \, (L_{H_2})^{-1}$ for Pd/Pt and Pt cathodes, respectively. While these values were relatively high in comparison to those reported by Logan and co-workers [11], they were well below a minimum of 4.5–$5.5 \, W \, (L_{H_2})^{-1}$ required for water electrolysis [10,11], thus confirming that hydrogen production was, indeed, due to biocatalyzed degradation of organic matter.

5. Conclusions

This study demonstrated hydrogen production in a continuous flow electrically assisted MFC. A single chamber design with a gas-collection chamber allowed for increased volumetric efficiency of the cell so that hydrogen production reached $0.98 \, L_{STP} \, (L_A \, d)^{-1}$ at a voltage of 1.16 V. Simultaneously a substrate removal rate of $1.6 \, g \, COD \, (L_A \, d)^{-1}$ with effluent COD concentration below $100 \, mg \, L^{-1}$ was achieved, i.e. hydrogen production was successfully combined with COD removal.

The MFC used a Pd/Pt cathode catalyst to facilitate the hydrogen reduction reaction. Pd has a high catalytic activity for hydrogen evolution reaction [18]. Furthermore, Pd is almost four times less expensive than Pt and even partial replacement of Pt by Pd significantly decreases the high cost associated with the catalyst. In future experiments Pd/Pt ratio might be optimized and non-noble catalysts might be tested.

Material balance calculations underlined the presence of a mixed microbial community in the MFC. In addition to exoelectrogenic microorganisms, acetoclastic and hydrogenotrophic methanogens were present. Consequently, a significant amount of carbon source was used by the acetoclastic methanogens, which competed with the exoelectrogenic microorganisms for a common substrate. Furthermore, hydrogenotrophic methanogens converted up to 50% of the hydrogen produced at the cathode into

methane. Biocatalyzed production of hydrogen from organic wastes requires a mixed consortium of anaerobic microorganisms capable of hydrolysis and fermentation of complex organic molecules. This justifies MFC inoculation with anaerobic sludge, which contains a broad range of fermentative microorganisms. However, activity of both acetoclastic and hydrogenotrophic methanogens should be restricted in order to increase the efficiency of hydrogen production. This can be accomplished by removing methanogenic microorganisms from the inoculum (i.e. by sludge pretreatment) and/or creating conditions unfavorable for methanogenic activity, i.e. by reducing pH of the anodic chamber.

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