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ARTICLE *in* ENERGY & FUELS · SEPTEMBER 2009

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Acoustics, Speech, and Signal Processing Newsletter, IEEE, 23, 9, 2009

Web page / page Web
http://dx.doi.org/10.1021/ef900357y
http://nparc.cisti-icist.nrc-cnrc.gc.ca/npsi/ctrl?action=rtdoc&an=13001058&lang=en
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Energy Fuels **2009,** *23,* 4612–4618 · DOI:10.1021/ef900357y
Published on Web 07/29/2009

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Hydrogen Production from Glycerol in a Membraneless Microbial Electrolysis Cell

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Received April 22, 2009. Revised Manuscript Received July 14, 2009

Hydrogen production from glycerol was studied in a microbial electrolysis cell (MEC) with a 250 mL anodic chamber and a gas-phase cathode. A membraneless MEC design was employed, where a graphite felt anode and gas diffusion cathode were only separated by a 0.7 mm thick highly porous synthetic fabric (J-cloth). Glycerol (fuel) was continuously fed to the anodic chamber at loads of 0.3−5.3 g L_a^{-1} d^{-1}. Fast conversion of glycerol to fermentation products, mainly 1,3-propanediol, propionate, and acetate was observed, that is, the fermentation products rather than glycerol were the most likely source of electrons for the anodophilic microorganisms. Hydrogen formation at the cathode required additional input of energy, which was provided by a controllable power supply. Hydrogen formation was observed starting from an applied voltage of 0.5 V. The highest volumetric rate of hydrogen production was 0.6 L L_a^{-1} d^{-1}, which was obtained at a glycerol load of 2.7 g L_a^{-1} d^{-1} and an applied voltage of 1.0 V. Hydrogen yield reached 5.4 mol per mol glycerol consumed, which corresponded to 77% of the theoretical value.

1. Introduction

Glycerol is the principal coproduct of biodiesel production. Crude glycerol derived from the biodiesel production process has many impurities, which decrease its commercial value. At the same time, glycerol purification to food or cosmetic usage grade is costly. The recent worldwide increase in biodiesel production has generated a glycerol surplus, resulting in a drop in glycerol prices. This means that glycerol produced during biodiesel production has become a "waste-stream" with a disposal cost associated to it.[1]

Several technologies based on chemical transformation of glycerol into more valuable products, mainly 1,3-propanediol[1−3] and hydrogen,[4−6] have been proposed. Although hydrogen yield in the pyrolitic decomposition of glycerol can almost reach its theoretical maximum of 7 mol_{H_2} mol^{-1} glycerol,[5,6] this technology requires high process temperatures, thus leading to significant energy losses. Recently, hydrogen production from glycerol−water solution in a PEM electrolysis cell has been proposed.[7] In this process, electrochemical reforming of glycerol was achieved using Pt/Ru−Ir oxide. Electrical energy consumption of 1.1 kWh m^{-3} of H_2 was reported and a volumetric hydrogen production rate of up to 10 $m_{H_2}{}^3$ m^{-3} d^{-1} was projected based on current measurements. However, this process required the use of noble catalysts at the anode, and poisoning of anode catalytic activity by glycerol or its oxidation products has been observed.

In general, hydrogen production from glycerol via biological fermentation process is less energy intensive. Biohydrogen production can be achieved via fermentative processes involving bacteria of the genera *Klebsiella*, *Citrobacters*, *Enterobacter*, and *Clostridia*.[1] Liu et Fang[8] demonstrated fermentative hydrogen production from glycerol with a maximum hydrogen evolution rate of 0.4 L_{H_2} L^{-1} h^{-1} using *Klebsiella pneumoniae* DSM 2026. Ito et al.[9] used *Enterobacter aerogenes* on porous ceramics as a support material, obtaining a hydrogen production rate of 1.4 L_{H_2} L^{-1} h^{-1}. However, thermodynamic limitations of the fermentation process result in only partial conversion of glycerol and therefore low hydrogen yields below 1 mol_{H_2} $mol_{glycerol}^{-1}$,[8,10] due to the production of several metabolites such as 1,3-propanediol, ethanol, and volatile fatty acids.[11,12]

Microbially catalyzed electrolysis is a novel technology capable of converting organic matter into hydrogen in a modified microbial fuel cell (MFC).[13] In a MFC, the anodophilic microorganisms convert chemical energy of organic matter (fuel) to electricity by transferring electrons to the anode and releasing protons, while oxygen reduction occurs at the cathode electrode.[14−16] In the microbial electrolysis cell

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(MEC) no oxygen is provided to the cathode and protons are reduced to molecular hydrogen provided that additional energy is supplied by an external power supply.[17−20] A detailed description of hydrogen production in a MEC can be found elsewhere.[13,18,19]

So far, hydrogen production in a MEC has been demonstrated on several volatile fatty acids, glucose, and cellulose.[20−24] However, thus far only results on hydrogen production from glycerol in a batch-fed MEC have been reported.[25] This study is aimed at demonstrating hydrogen production from glycerol in a membrane-less MEC with a gas-phase cathode, which was shown to improve the volumetric rate of hydrogen production in comparison with a PEM or liquid-phase cathode MECs.[20,26]

2. Materials and Methods

2.1. Media Composition. The stock solution of carbon source contained 265.0 g L^{-1} of glycerol. The nutrients stock solution was composed of (in g L^{-1}): yeast extract (0.83), 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 Rozendal et al.[21] and contained (in mg L^{-1}) $FeCl_2 \cdot 4H_2O$ (2000), H_3BO_3 (50), $ZnCl_2$ (50), $CuCl_2$ (30), $MnCl_2 \cdot 4H_2O$ (500), $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ (50), $AlCl_3$ (50), $CoCl_2 \cdot 6H_2O$ (50), $NiCl_2$ (50), EDTA (500), and HCl (1 mL). All solutions were filter sterilized and stored at 4 °C to prevent microbial growth. 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, USA) 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. The 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.

1,3-Propanediol was analyzed on a gas chromatograph (6890 Series, Hewlett-Packard, Wilmington, DE) coupled to an FID detector. A 1 μL portion of water sample was injected on a DB-ACL2 capillary column of 30 m × 530 μm × 2 μm from Agilent Technologies (Wilmington, DE, USA) The column was heated at 60 °C for 2 min and then raised to 190 °C at a rate of 10 °C/min. Helium was used as carrier gas. The injector and detector were maintained at 240 and 250 °C, respectively.

Glycerol was measured by HPLC (Waters Corp, Milford, MA, USA) using model 717 Plus equipped with an autosampler, a refractive index detector (Waters model 2414) and a PDA detector (model 2996). Transgenomic ICSep IC-ION-300 (300 mm × 7.8 mm OD) HPLC column was used. The mobile phase was 0.01N H_2SO_4 at 0.4 mL min^{-1}. Analysis was carried out at 35 °C. Standard deviations of all analytical methods did not exceed 5%.

Gas production in the MEC was measured online using bubble counters connected to glass U-tubes and interfaced with a data acquisition system.[20] The U-tubes contained a dye, which facilitated bubble counting. Gas composition was measured using a gas chromatograph (6890 Series, Hewlett-Packard, Wilmington, DE) equipped with a 3.5 m × 2 mm i.d. Chromosorb 102 column (Supelco, Bellefonte, PA, USA) and a thermal conductivity detector. The column was heated at 50 °C for 4 min. The carrier gas was argon.

2.3. Electrochemical Measurements and Calculations. In hydrogen-production mode (MEC), an adjustable DC power supply (IF40GU Kenwood, Japan) was used to maintain voltage at the preset set point. In electricity-production mode (MFC), voltage was measured online at 10 min intervals using a data acquisition system (Labjack U12, Labjack Corp, Lakewood, CO, USA). In MEC mode, voltage scans were carried out by changing the applied voltage from 1.2 to 0.4 V in 0.2 V steps. Once the voltage setting was changed, current was measured after 10 min using a multimeter (Fluke 189, Fluke Corp, Everett, WA, USA). MEC internal resistance (i.e., the sum of the charge transfer resistances and the solution resistance) was estimated using the linear part of the voltage scan. Anode potential at each voltage was measured using a standard calomel electrode (SCE, 0.2412 V vs NHE).

Following previous reports,[17−21,27] MEC performance was evaluated in terms of hydrogen yield from glycerol, specific energy consumption, energy efficiency (the amount of energy contained in hydrogen as compared to the power input necessary to produce this amount of hydrogen), as well as in terms of Coulombic efficiency, cathodic efficiency, and chemical oxygen demand (COD) removal efficiency. Detailed explanations of the calculation methods for these parameters are provided below.

Hydrogen yield on glycerol (Y_{H_2}, $mol_{H_2}\, mol_g^{-1}$) was calculated as

$$Y_{H_2} = \frac{(p \cdot Q_{H_2} V_a)/(RT)}{(G_{in} - COD_{out}/r_g)/M_g Q_{in}} \quad (1)$$

where p is the pressure (p = 1 atm); Q_{H_2} is the hydrogen flow rate ($L_{H_2}\, L_a^{-1}\, d^{-1}$); V_a is the anode volume (V_a = 0.25 L); R is the ideal gas constant (R = 0.08205 L atm K^{-1} mol^{-1}); T is the temperature (T = 298 K); G_{in} is the concentration of glycerol in the influent (g L^{-1}); COD_{out} is the COD of the effluent (g L^{-1}); r_g is the COD equivalent of 1 g of glycerol (r_g = 1.2 g g^{-1}); M_g is the molecular weight of glycerol (M_g = 92.09 g mol^{-1}); and Q_{in} is the influent flow rate (L d^{-1}).

Specific energy consumption (E_{cons}, Wh $L_{H_2}^{-1}$) was calculated as:

$$E_{cons} = \frac{\int_0^{86400} E_{app} I \, dt}{(Q_{H_2} V_a) 3600} \quad (2)$$

where E_{app} is the voltage applied to MEC (V) and I is the current (A).

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Table 1. Influent End Effluent Composition in Glycerol Load Tests[a]

	influent	effluent and off-gas						
OLR g L_a^{-1} d^{-1}	glycerol (mg/L)	acetate (mg/L)	propionate (mg/L)	butyrate (mg/L)	glycerol (mg/L)	propanediol (mg/L)	hydrogen (mg/day)	COD recovery (%)
5.3	1433.2	281.2	17.5	83.6	7.1	304.0	5.9	59.3
2.7	743.6	103.0	178.1	52.8	1.8	56.0	12.7	69.8
1.3	397.7	32.8	33.8	38.4	0.0	25.0	9.6	50.8
0.7	209.1	14.7	45.7	17.4	1.7	13.5	8.6	72.9
0.3	106.6	5.0	3.8	0.0	1.9	0.0	5.5	32.6
2.7[b]	743.6	116.1	176.3	58.4	0.0	59.0	0.0	64.8

[a] Unless specified, tests were carried out at an applied voltage of 1.0 V. COD recovery is calculated by comparing COD equivalents of glycerol fed to MEC (in g day^{-1}) with the sum of COD equivalents of all measurable products in the liquid (glycerol, acetate, propionate, butyrate, propanediol) and gas (hydrogen, methane) phases. [b] No applied voltage.

Coulombic efficiency (ε_C) was calculated as the ratio between the total Coulombs actually transferred to the anode from the substrate to the anode, and the theoretical maximum:[19]

$$\varepsilon_C = \frac{\int_0^{86400} I\,dt}{(G_{in} - COD_{out}/r_g)/M_g Q_{in} e_g F} 100\% \quad (3)$$

where e_g is the number of mol of electrons exchanged per mol of glycerol equivalent consumed (14 mol mol^{-1}), and F is the Faraday constant (96 485 C mol^{-1}).

Cathodic efficiency (ε_C), was calculated as the ratio of hydrogen recovery in the cathode to maximum possible if all the current is converted to hydrogen:

$$\varepsilon_C = \frac{[(pQ_{H_2}V_a)/(RT)]e_{H_2}F}{\int_0^{86400} I\,dt} 100\% \quad (4)$$

where e_{H_2} is the number of mol of electrons exchanged per mol of hydrogen (2 mol mol^{-1}).

Energy efficiency (ε_E) was calculated as the amount of energy recovered as hydrogen compared to the power input necessary to produce this amount of hydrogen:

$$\varepsilon_E = \frac{[(pQ_{H_2}V_a)/(RT)]\Delta G^\circ r_{H_2O}}{\int_0^{86400} E_{app} I\,dt} 100\% \quad (5)$$

where $\Delta G^\circ r_{H_2O}$ is the Gibbs free energy of hydrogen combustion (equal to water formation, $\Delta G^\circ r_{H_2O} = -237$ kJ/mol). Notably, Gibbs free energy of glycerol is not considered in eq 5, hence ε_E could reach values higher than 100%.

COD removal efficiency (ε_{COD}) is defined as the ratio between the effluent and influent concentrations of glycerol and degradation products expressed in COD equivalents:

$$\varepsilon_{COD} = \frac{(G_{in} r_g - COD_{out})}{G_{in} r_g} 100\% \quad (6)$$

where G_{in} is the influent glycerol concentration (mg L^{-1}) and COD_{out} is the sum of effluent concentrations of glycerol and all measurable degradation intermediates expressed in COD equivalents (mg L^{-1}).

2.4. MEC Design, Instrumentation, and Operation. All experimentation was carried out in a continuous-flow MEC constructed with a series of polycarbonate plates arranged to form an anodic chamber and a gas collection chamber as described elsewhere.[20,26] The anodic chamber retained 210 mL of liquid and had a headspace of 40 mL. The gas collection (cathodic) chamber also had a volume of 250 mL.

Graphite felt, 5 mm thick, measuring 25 × 10 cm (Speer Canada, Kitchener, ON, Canada) was placed in the anodic chamber filled with liquid. 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) was used as a cathode. The cathode was separated from the anode by a piece of porous cellulosic nonwoven fabric (J-cloth) with a thickness of 0.7 mm. The MEC was inoculated with 25 mL of heat-treated (20 min at 100 °C) homogenized anaerobic sludge (Lassonde Inc., 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 0−5 mL d^{-1}. A 1 mL portion of trace metals stock solution and 42 mL of nutrients solution were added to 1 L of the dilution water. The dilution water was fed at a rate of 750 mL d^{-1} using a peristaltic pump (Cole-Parmer, Chicago, IL, USA) providing a retention time of 8 h. Mixing in the anodic chamber was provided by an external recirculation loop. A recirculation rate of 1.44 L h^{-1} was used.

MEC 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 × 10 cm heating plate located on the anodic chamber side of the MEC. The 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 into the recirculation line.

MEC performance during hydrogen production from glycerol was evaluated using several techniques. First, the amount of glycerol fed to the MEC was optimized in glycerol load tests, where a preset value of applied voltage was maintained while periodically changing the glycerol load (Table 1). Next, the dependence of hydrogen production on applied voltage was studied in applied voltage tests, where the load of glycerol was maintained at a constant level while the voltage was varied (Table 2). Each set of operating conditions was maintained for at least 2 days (6 retention times), except a glycerol load of 5.3 g L_a^{-1} d^{-1}, which was maintained for 7 days. At the end of each test, the hydrogen production rate was estimated by averaging the measurements obtained during last 8 h of the test.

3. Results

3.1. Start-up Procedure. After anodic chamber inoculation with heat-treated anaerobic sludge, the cell was fed with acetate and operated in electricity production (MFC) mode by exposing the gas-collection chamber to air. Anode and cathode electrodes were externally connected through a 400 Ω resistor and the potential was continuously measured, thus permitting online monitoring of the anode colonization

Table 2. Influent and Effluent Composition in Applied Voltage Tests[a]

	influent	effluent and off-gas						
V_{app} (V)	glycerol (mg/L)	acetate (mg/L)	propionate (mg/L)	butyrate (mg/L)	glycerol (mg/L)	propanediol (mg/L)	hydrogen (mg/day)	COD recovery (%)
1.0	209.1	14.7	45.7	17.4	1.7	13.5	8.6	72.9
0.7	209.1	17.1	8.7	0.0	0	14.2	8.4	38.9
0.5	209.1	13.8	16.3	0.0	0	0.0	6.1	27.9
0.0	209.1	36.0	16.3	0.0	0	11.2	0.0	31.0

[a] All tests were carried out at a glycerol load of 0.7 g L_a^{-1} d^{-1}.

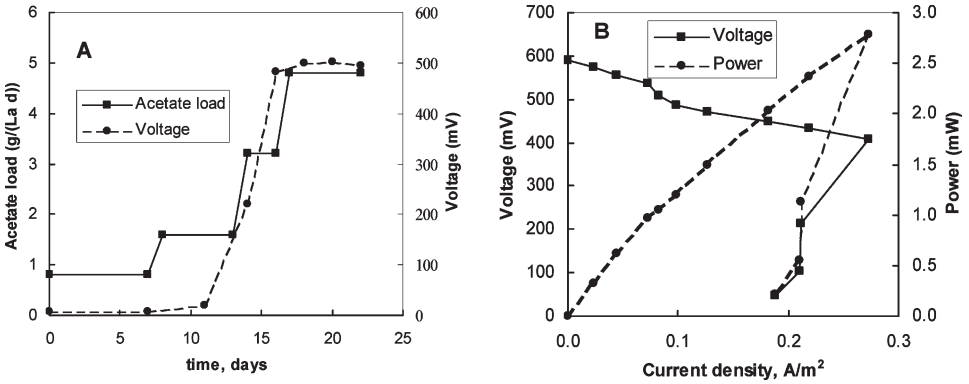

Figure 1. (A) Acetate load and resulting voltage during the start-up process in MFC mode. MFC was operated at R_{ext} = 400 Ω and a temperature of 25 °C. (B) Polarization and power curves obtained at the end of the start-up period (day 18).

process. Initially, an acetate load of 0.9 g L_a^{-1} d^{-1} was used, then the acetate load was gradually increased to 4.8 g L_a^{-1} d^{-1} as shown in Figure 1A. This startup strategy was aimed at providing a sufficient amount of substrate while avoiding excessive levels of acetate in the anodic chamber, which could inhibit microbial activity (i.e., organic overload conditions). The choice of acetate as the initial carbon source enabled a comparison of MEC performance on acetate and glycerol. After 10 days of operation the potential started to increase steadily, and 10 days later it stabilized at 490 mV. An open circuit voltage of 589 mV was measured. At the end of the start-up process (day 18) a polarization test was conducted by gradually decreasing the external resistance. On the basis of the linear section of the polarization plot, an internal resistance of 17.2 Ω was calculated. However, a maximum power of 2.78 mW (11.2 mW L_a^{-1}) was obtained at a higher resistance of 40 Ω. A sharp drop in MFC performance was observed below an external resistance of 40 Ω as shown in Figure 1B, likely due to mass transfer limitations of the carbon source and its degradation products.[28]

Hydrogen production mode was initiated by flushing the gas-collection (cathode) chamber with pure nitrogen and applying an external voltage of 1 V, while continuing to feed MEC with acetate. Hydrogen production began almost immediately. After 2 days of operation, a current density of 77 mA (3.08 A m^{-2}) was observed corresponding to a hydrogen production rate of 4.34 ± 0.76 L_{H_2} L_a^{-1} d^{-1}. After subtracting background current as described below, a power consumption of 1.71 ± 0.23 Wh $L_{H_2}^{-1}$ was estimated. Gas-collection (cathodic) chamber off-gas consisted of H_2 (96.6%), N_2 (0.3%), and water vapor (3.1%). Methane was not detected, and no gas production was observed in the anodic chamber. Two days after hydrogen production mode was initiated the carbon source was changed from acetate to glycerol, which was fed at a rate of 5.3 g L_a^{-1} d^{-1}. Changing the carbon source from acetate to glycerol led to a decrease in hydrogen production from 4.34 ± 0.76 to 0.29 ± 0.03 L_{H_2} L_a^{-1} d^{-1}. This drop in hydrogen production was accompanied by an increase in power consumption (from 1.7 ± 0.23 to 4.29 ± 0.50 Wh $L_{H_2}^{-1}$) and an accumulation of 1,3-propanediol and other fermentation products.

3.2. Glycerol Load Tests. The glycerol load tests were aimed at optimizing the amount of glycerol fed to the MEC. An initial verification of hydrogen production in the absence of glycerol was carried out at an applied voltage of 1.0 V. In this test MEC was fed with the solution containing no glycerol, while all other medium components were retained. No hydrogen production was detected in this case, even though a background current density of 0.36 A m^{-2} was measured. This background current was attributed to redox processes associated with salts in the medium. To confirm that the background current is not related to microbial activity, a second MEC was assembled and operated for 48 h abiotically, that is, in the absence of a anodophilic microbial inoculum at the anode. This MEC was filled with the standard solution of salts and nutrients, except no glycerol was added. Once again, at an applied voltage of 1.0 V no hydrogen production was detected but a current density of 0.32−0.36 A m^{-2} was measured. A voltage scan showed that at 0.75 V the background current density decreased to 0.04 A m^{-2}, and at 0.5 V no measurable current was detected. Thus, measurements of background current obtained in the absence of glycerol represent nonbiological reactions, such as electrochemical reactions due to high concentration of salts in the medium. In all subsequent calculations the measurements at each applied voltage were corrected with respect to these background measurements.

MEC was operated at several glycerol loads ranging from 0.3 to 5.3 g L_a^{-1} d^{-1}. Glycerol fed to MEC was readily transformed to fermentation products, mainly 1,3-propanediol and acetate (Table 1). At a glycerol load of 5.3 g L_a^{-1} d^{-1}, high concentrations of all fermentation products found in

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the anodic chamber effluent suggested organic overload. Analysis of hydrogen production rates and hydrogen yields given in Figure 2A show that glycerol overload led to a decrease in the rate of hydrogen production (0.29 ± 0.03 L_{H_2} L_a^{-1} d^{-1}) with a corresponding hydrogen yield of only 0.49 ± 0.06 mol_{H_2} mol_g^{-1}.

When glycerol load was decreased to 2.7 g L_a^{-1} d^{-1} the concentration of metabolites in the effluent decreased with the exception of propionic acid, which had increased 10 times (Table 1). Hydrogen production doubled to 0.62 ± 0.04 L_{H_2} L_a^{-1} d^{-1}, whereas current remained almost unchanged, thus leading to a 50% decrease in specific energy consumption (Figure 2A). Also, hydrogen yield increased 5-fold and Coulombic efficiency improved from 13 to 34%. At glycerol loads of 1.3 and 0.7 g L_a^{-1} d^{-1} hydrogen production rates were 0.47 ± 0.05 and 0.42 ± 0.07 L_{H_2} L_a^{-1} d^{-1}, respectively. Specific energy consumption values (2.86 ± 0.30 and 2.97 ± 0.42 Wh $L_{H_2}^{-1}$, respectively) were similar to those observed at a glycerol load of 2.7 g L_a^{-1} d^{-1} (Figure 2A). A further decrease in the glycerol load to 0.7 g L_a^{-1} d^{-1} improved the Coulombic efficiency as shown in Figure 2B. Also, hydrogen yield increased from 2.26 ± 0.27 to 5.39 ± 0.90 mol_{H_2} mol_g^{-1} (Figure 2A). However, at a much reduced glycerol load of 0.3 g L_a^{-1} d^{-1}, hydrogen production was found to decrease and energy consumption went up. Hydrogen yield and Coulombic efficiency also decreased considerably. At all glycerol loads current density was between 0.89 and 0.92 A m^{-2}. Notably, Coulombic efficiency was low at high glycerol loads suggesting the existence of unidentified intermediates of glycerol degradation at high loads. Glycerol degradation (i.e., mineralization) efficiency was up to 90% at the lowest glycerol load but remained below 40% at glycerol loads above 1.3 g L_a^{-1} d^{-1} (Figure 3A) due to high concentrations of degradation intermediates in the effluent as shown in Table 1.

3.3. Applied Voltage Tests. To investigate the effect of the applied voltage on MEC performance, the cell was operated at three different voltages of 1.0, 0.75, and 0.5 V and in the fermentation mode, that is, when no voltage was applied to MEC. During these tests glycerol load was always maintained at 0.7 g L_a^{-1} d^{-1} to avoid accumulation of glycerol degradation products. As can be seen from a comparison of hydrogen production rates shown in Figure 4A, between 0.5 and 0.75 V the hydrogen production rate increased with increasing voltage, however energy consumption per L of produced hydrogen also increased. Current density changed from 0.64 to 0.72 A m^{-2} when the applied voltage was increased from 0.5 to 0.75 V. Above 0.75 V the hydrogen production reached a plateau and the current density only slightly increased to 0.88 A m^{-2}. Also, the Coulombic efficiency improved with increasing voltage (Figure 4B).

Glycerol removal efficiency remained between 69 and 84% at applied voltages of 0.5 and 0.75 V, then it dropped to 44% at 1.0 V (Figure 3B). The main metabolites found in the effluent were acetate, propionate, and 1,3-propanediol. Butyrate only appeared when the applied voltage was set at 1.0 V. A detailed composition of the anodic chamber effluent is provided in Table 2. When no voltage was applied to MEC, thus limiting activity of anodophilic microorganisms, acetate and butyrate concentration in the effluent increased 11 and 9%, respectively, while concentrations of other metabolites only varied within ±4% .

Both in glycerol load and applied voltage tests, no net gas production was measured in the anodic chamber. However, headspace analysis showed the appearance of methane after the first 20 days of MEC operation. During the last week of MEC operation, which lasted 57 days, up to 2% of methane

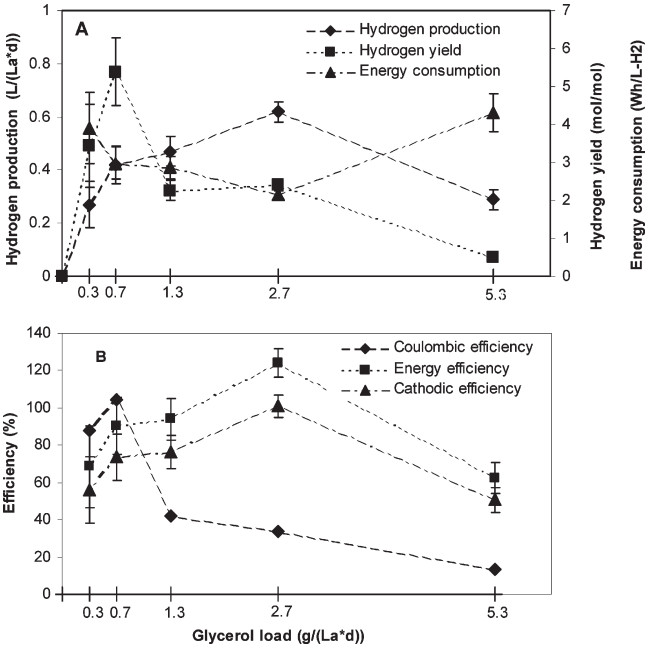

Figure 2. Dependence of (A) hydrogen production rate, hydrogen yield, specific energy consumption and (B) hydrogen production efficiency (Coulombic, cathodic, and energy efficiency) on glycerol load. All tests were carried out at an applied voltage of 1.0 V. Energy efficiency calculation (eq 5) does not consider Gibbs free energy of glycerol, thus leading to values above 100%.

was found in the hydrogen stream. Apparently, this methane diffused from the anodic chamber where it was produced by methanogenic microorganisms, which survived the heat treatment procedure.

3.4. Electrochemical Performance. The electrochemical performance of the MEC was evaluated by measuring the anode potential and internal resistance at the end of each test. Results of anode potential measurements are shown in Figure 5A. The highest anode potentials were observed at the lowest glycerol load (0.3 g L_a^{-1} d^{-1}), and the lowest values were obtained at glycerol loads of 2.7 and 1.3 g L_a^{-1} d^{-1}. Cathode potential, computed as the difference between the anode potential and applied voltage, remained almost constant at all voltages. It was estimated at -621 ± 77 mV versus NHE.

Calculation of MEC internal resistances based on the results of the voltage scan tests shown in Figure 5B yielded values between 18 and 100 Ω. Interestingly, the highest internal resistance was obtained when the glycerol load was low or high, and the lowest values once again corresponded to glycerol loads of 2.7 and 1.3 g L_a^{-1} d^{-1}, that is, when hydrogen production was the highest. Measurements of anodic liquid conductivity showed no difference between the tests conducted at different glycerol loads and different applied voltages. The conductivity always remained at 15–16 mS cm^{-1} due to the high ionic strength of the phosphate buffer.

4. Discussion

4.1. Hydrogen Formation from Glycerol. Microbially catalyzed production of hydrogen from glycerol is described by the following reaction:

$$C_3H_8O_3 + 6H_2O \rightarrow 3HCO_3^- + 3H^+ + 7H_2 \qquad (7)$$

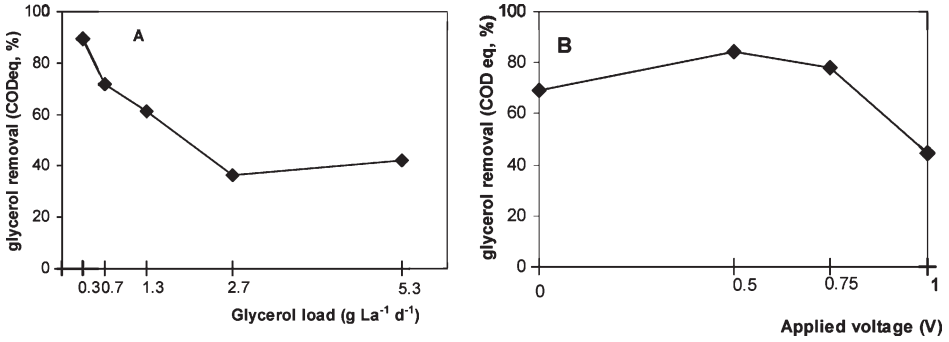

Figure 3. Glycerol removal efficiencies (expressed in COD equivalents) at different glycerol loads (A) and different applied voltages (B). In glycerol load tests voltage was maintained at 1.0 V. In applied voltage tests glycerol load was maintained at 0.7 g L_a^{-1} d^{-1}.

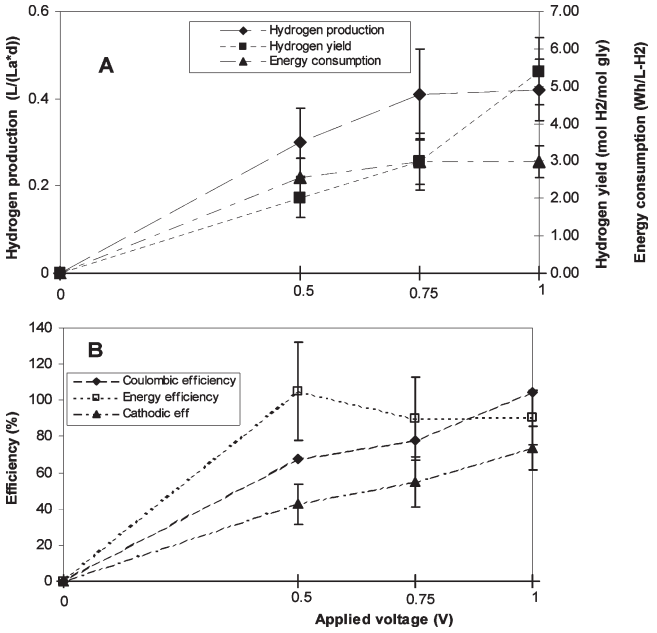

Figure 4. Dependence of (A) hydrogen production rate, hydrogen yield, specific energy consumption and (B) Coulombic, energy, and cathodic efficiencies on applied voltage. Results were obtained at a glycerol load of 0.7 g L_a^{-1} d^{-1}. Energy efficiency calculation (eq 5) did not include Gibbs free energy of glycerol.

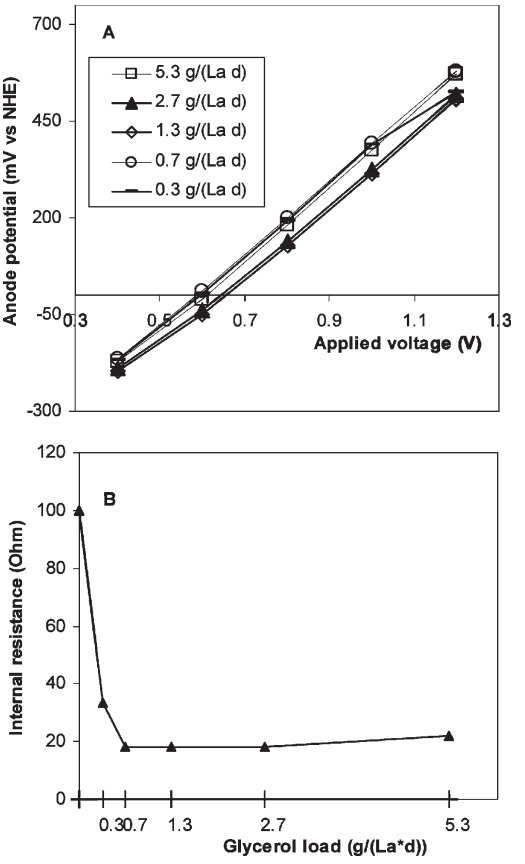

Figure 5. Electrochemical characterization of MEC showing (A) anode potentials and (B) dependence of internal resistance on glycerol load.

which indicates a yield of 7 mol of hydrogen per mol of glycerol. Accordingly, the anodic half-reaction of glycerol oxidation is given by

$$C_3H_8O_3 + 6H_2O \rightarrow 3HCO_3^- + 17H^+ + 14e^- \quad (8)$$

and the cathodic half-reaction of hydrogen formation is

$$2H^+ + 2e^- \rightarrow H_2 \quad (9)$$

Calculation of the anode and cathode potentials at pH 7 according to the Nernst equation (assuming 20 mM of glycerol, 2 mM of bicarbonate, and a hydrogen partial pressure of 1 atm) results in values of −425 mV and −414 mV versus NHE (normal hydrogen electrode), respectively. Since the potential at the anode is lower than the potential at the cathode, it theoretically suggests that hydrogen production from glycerol at pH 7 does not require an additional input of energy. In contrast, hydrogen production from acetate requires an energy input of 104.6 kJ/mol, which corresponds to an applied voltage of 0.14 V.[18,19] However, the experimental results presented above provide no evidence of direct glycerol oxidation at the anode by anodophilic microorganisms since hydrogen production was not observed below an applied voltage of 0.5 V. An energy input of at least 2.17 W h $L_{H_2}^{-1}$ was required to achieve hydrogen formation from glycerol, which could be explained by energy losses due to electrode overpotentials, glycerol consumption by fermentative rather than anodophilic microorganisms, and production of several metabolites. Indeed, analysis of the anodic chamber effluent given in Table 1 shows the presence of several fermentation products, namely, acetate, propionate, butyrate, and 1,3-propanediol. These fermentation products, rather than glycerol, were likely used by the anodophilic microorganisms.

Moreover, it appeared that not all fermentation products were consumed by the anodophilic microorganisms. Table 1 describes fermentation products found in the anodic chamber effluent at an applied voltage of 1.0 V and when no voltage was applied to the MEC (fermentation mode). A comparison

shows similar concentrations of 1,3-propanediol, propionate, and butyrate, whereas acetate concentration is lower when voltage was applied. It can be hypothesized that acetate was the preferred carbon source for anodophilic microorganisms. Nevertheless, consumption of other fermentation products can not be excluded without further investigation. Also, material balance calculations showed COD recovery in a range of 50−70% (Table 1), which suggests that not all metabolites of glycerol fermentation (i.e., succinic acid, 1,2-propanediol, isopropanol-amine[12]) were accounted for by the analytical procedures used in this study.

Electrode overpotentials also contributed to the relatively high energy consumption observed throughout the experiments. Anode potentials measured during the voltage scans (Figure 5A) were between −193.8 mV versus NHE (glycerol load = 2.7 g L_a^{-1} d^{-1}; applied voltage = 0.4 V) and 697 mV versus NHE (glycerol load = 0.0 g L_a^{-1} d^{-1}; applied voltage = 1.2 V), which corresponded to anode overpotentials between 222 and 996 mV, respectively. High anode overpotential at 1.2 V might explain the high power requirement at this voltage. As a result, energy efficiency declined at applied voltages above 0.5 V (Figure 4B).

4.2. Glycerol Load and Applied Voltage Optimization. MEC operation at different glycerol loads clearly demonstrated the importance of this operational parameter in optimizing process performance. When glycerol loads were low, the concentration of degradation intermediates detected in the anodic chamber effluent was insignificant, indicating a high glycerol removal efficiency. However, a comparison of Figures 2A and 3A shows that the volumetric rate of hydrogen production was low. By increasing the glycerol load to 2.7 g L_a^{-1} d^{-1} the hydrogen production increased, but the concentrations of fermentative products, in particular 1,3-propanediol, also increased and glycerol removal efficiency declined. Further increase in the glycerol load to 5.3 g L_a^{-1} d^{-1} led to a sharp decrease in hydrogen production, coinciding with an increase in specific energy consumption (Figure 2A). Also, a glycerol load of 2.7 g L_a^{-1} d^{-1} corresponded to the lowest specific energy consumption, which was estimated at 2.17 Wh $L_{H_2}^{-1}$. Hydrogen yield and Coulombic efficiency exhibited a similar trend, but the best values were obtained at a lower glycerol load of 0.7 g $L_a^{-1}d^{-1}$.

Interestingly, MEC internal resistance was found to be dependent on the glycerol load such that the lowest internal resistance was measured at 2.7 g L_a^{-1} d^{-1} (Figure 5B). This glycerol load also corresponded to the lowest anode overpotential (Figure 5A), suggesting a link between the activity of anodophilic microorganisms and the electrochemical properties of the MEC. Overall, the MEC performance was considered most efficient at a glycerol load of 2.7 g L_a^{-1} d^{-1}, but this value might be dependent on MEC design, microbial populations, influent composition, and other factors. A feedback control system that adjusts glycerol load in response to varying operational conditions can be used to maximize MEC performance and should be considered for future research.

In addition to the formation of 1,3-propanediol at the anode, the possibility of 1,3-propanediol production by electrochemical reduction of glycerol at the cathode was hypothesized. However, 1,3-propanediol concentrations in MEC effluent were similar when no voltage was applied and at 1.0 V (Table 1, a glycerol load of 2.7 g L_a^{-1} d^{-1}). Also, no 1,3-propanediol formation was observed in the abiotic control MEC fed with glycerol and operated at 1.0 V. Thus, 1,3-propanediol was produced from glycerol by fermentative microorganisms rather than electrochemically, which is in agreement with the reductive pathway of glycerol biotransformation by anaerobic microorganisms, which has been reported to result in 1,3-propanediol formation at high glycerol loads.[29] This might explain the 6-fold increase of 1,3-propanediol observed in the experiment when the glycerol load was increased (Table 1).

The results of MEC operation at several voltages with a constant glycerol load of 0.7 g L_a^{-1} d^{-1} demonstrated the highest energy efficiency at 0.5 V (Figure 4). Glycerol removal efficiency significantly declined when applying voltages above 0.75 V (Figure 3B). Nevertheless, since the hydrogen production rate and yield were highest at an applied voltage of 1 V, MEC operation at 0.75 V can be suggested as a compromise between energy efficiency and volumetric performance objectives.

5. Conclusion

Results of this study demonstrated the feasibility of hydrogen production by microbially catalyzed electrolysis of glycerol. Microbial electrolysis of glycerol resolves the thermodynamic limitations associated with dark fermentation, thus resulting in almost complete conversion of organic matter to hydrogen.[18,19] The rate of hydrogen production from glycerol observed in a MEC was comparable to that observed in the glycerol fermentation process.[8,9] However, the hydrogen yield was significantly higher and reached 5.39 mol_{H_2} mol_g^{-1} as opposed to yields below 1 mol H_2 mol^{-1} glycerol reported in the literature.[8,10] Although relatively high power inputs (2−3 Wh $L_{H_2}^{-1}$) were required when using glycerol, these values were always below a minimum of 5 Wh $L_{H_2}^{-1}$ required for hydrogen production by water electrolysis.[19] Power inputs below 1 Wh $L_{H_2}^{-1}$ might be expected if anode overpotential could be decreased, that is, through improved electrode materials.[20] Also, a decreased power consumption and an improved volumetric hydrogen production rate can be expected if operating conditions are further optimized or an acidification step is added to ensure glycerol conversion to volatile fatty acids. Notably, a volumetric rate of up to 6 L L_a^{-1} d^{-1} was observed in the acetate-fed MEC[26] while a power consumption as low as 0.6 Wh $L_{H_2}^{-1}$ was observed at low applied voltages.[19] Furthermore, selection of anodophilic microorganisms capable of direct glycerol utilization might also improve MEC volumetric performance and further decrease the required power input.

Acknowledgment. This is NRC publication No. 49973.

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