High hydrogen production from glycerol or glucose by electrohydrogenesis using microbial electrolysis cells

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\section*{1. Introduction}

Glycerol is a commodity chemical widely used by the pharmaceutical industry. However, it is being overproduced as a result of biodiesel fuel production as 1 L of glycerol is made for every 10 L of biodiesel fuel produced. At the current annual production capacity of 9.8 billion liters (www.biodiesel.org), 980 million liters of glycerol/yr are produced compared to a demand of only 216 million liters/yr [1].

One alternative use for glycerol is hydrogen gas production by anaerobic fermentation [2–4]. However, only a maximum of 3 mol of H\textsubscript{2} can be produced per mole of glycerol if acetate is the main soluble fermentation end product. Further conversion to hydrogen without additional energy is not possible due to an overall endothermic reaction. Hydrogen yields obtained from pure glycerol (P-glycerol) fermentation are often substantially lower than this maximum value, mainly due to formation of 1,3-propanediol (PD), by a reaction which requires hydrogen [5]. Yields obtained during glycerol fermentation were 0.05–0.28 mol-H\textsubscript{2}/mol using mixed cultures [3,5], and 0.61–1.05 mol-H\textsubscript{2}/mol using pure cultures [2,4]. The actual glycerol byproduct from biodiesel (B-glycerol) produced

\section*{A B S T R A C T}

The use of glycerol for hydrogen gas production was examined via electrohydrogenesis using microbial electrolysis cells (MECs). A hydrogen yield of 3.9 mol-H\textsubscript{2}/mol was obtained using glycerol, which is higher than that possible by fermentation, at relatively high rates of 2.0 \pm 0.4 m\textsuperscript{3}/m\textsuperscript{3} d (E_{ap} = 0.9 V). Under the same conditions, hydrogen was produced from glucose at a yield of 7.2 mol-H\textsubscript{2}/mol and a rate of 1.9 \pm 0.3 m\textsuperscript{3}/m\textsuperscript{3} d. Glycerol was completely removed within 6 h, with 56% of the electrons in intermediates (primarily 1,3-propanediol), with the balance converted to current, intracellular storage products or biomass. Glucose was removed within 5 h, but intermediates (mainly propionate) accounted for only 19% of the electrons. Hydrogen was also produced using the glycerol byproduct of biodiesel fuel production at a rate of 0.41 \pm 0.1 m\textsuperscript{3}/m\textsuperscript{3} d. These results demonstrate that electrohydrogenesis is an effective method for producing hydrogen from either pure glycerol or glycerol byproducts of biodiesel fuel production.

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up to 0.31 mol-H₂/mol using mixed cultures and 1.12 mol-H₂/mol using pure cultures [2,5].

An alternative to glycerol fermentation for hydrogen production is the process of electrohydrogenesis using microbial electrolysis cells (MECs) [6,7]. In an MEC, exoelectrogenic bacteria oxidize organic matter and release electrons to the anode and protons into solution. A small electrical input (~0.2 V) is added, in addition to that supplied by the bacteria (~0.3 V anode open circuit potential for acetate) to overcome the endothermic barrier of hydrogen formation (0.414 V for acetate) [8]. With an MEC it is possible to achieve nearly stoichiometric conversion of a substrate to hydrogen. For example, 3.9 mol-H₂/mol-acetate (6.32 L/L d, applied 1 V) was obtained in a membraneless MEC [9] and 2.1 mol-H₂/mol-acetate (0.05 L/L d, applied 0.8 V) [10] was obtained in a two-chamber MEC with a proton exchange membrane compared to the stoichiometric limit of 4 mol-H₂/mol-acetate. The theoretical minimum electrical input needed is 0.12 V for acetate [8], but in practice a higher voltage is needed to overcome electrode overpotentials and to increase rates. MECs are especially useful when the substrate originates from a “waste product” such as wastewater [11].

The glycerol byproduct from biodiesel (B-glycerol) was used in a two-chamber MEC with a mediator, but the maximum yield was only 0.77 mol-H₂/mol-glycerol [12]. Glycerol has not yet been used in a mediatorless or membraneless MEC. Single-chamber membraneless systems with acetate have shown higher hydrogen production rates than systems with membranes due to reduced ohmic resistance and pH gradients in the system [9,13]. Also, membraneless systems are simpler to manufacture and they have reduced capital costs.

In this study, P-glycerol and B-glycerol were evaluated in a single-chamber mediatorless MEC. The goal was to obtain higher hydrogen yields than those obtained by fermentation, and therefore to achieve yields closer to the maximum theoretical yield of 7 mol-H₂/mol-glycerol by oxidation. Glucose was used as a positive control, as it is also a fermentable substrate and it shares similar stoichiometry and metabolic pathways as glycerol during bacterial degradation [3]. The intermediate product formation was examined over time to follow charge balances of these fermentable substrates. MEC performance and methane formation were also evaluated at different substrate concentrations and applied voltages for P-glycerol and B-glycerol.

2. Materials and methods

2.1. Substrates

P-glycerol (ultrapure) was obtained from MP Biomedicals, LLC (Solon, OH). Glucose (o-glucose, anhydrous) was obtained from J.T. Baker (Phillipsburg, NJ). B-glycerol was donated by Nittany Biodiesel (State College, PA). This B-glycerol, produced from the transesterification of soybean oil with methanol, sodium hydroxide and sodium methylate, had a chemical oxygen demand (COD) of 1160 ± 100 mg/L and a glycerol content of 69.5%.

2.2. MEC reactor construction and operation

Single-chamber membraneless MEC reactors consisted of a 4-cm long by 3-cm diameter cylindrical chambers formed from a solid block of Lexan, as developed by Call and Logan [13]. The anodes were ammonia-treated graphite brushes (25 mm diameter × 25 mm length, 0.22 m² surface area) [14,15]. The brush anodes were first enriched in microbial fuel cells (MFCs) with carbon cloth air cathodes [16] initially inoculated with domestic wastewater (first cycle only) and the substrate (1 g/L) to be used in MEC mode. On a molar basis, this represents a starting substrate quantity of 10.9 mM of P-glycerol, 7.52 mM of B-glycerol and 5.5 mM of glucose. The brush anodes remained in MFC cells until they reached three repeatable cycles and then they were transferred to MEC reactors. The MEC cathodes (surface area 7 cm²) were wet-proofed carbon cloth (B-1/B/30WP; BASF Fuel Cell, Somerset, NJ) with a platinum catalyst (10 wt% on Vulcan XC-72; BASF Fuel Cell, Somerset, NJ). The media for both MFC and MEC consisted of a 50 mM phosphate buffer solution (4.58 g/L Na₂HPO₄ and 2.45 g/L NaH₂PO₄, pH = 7.0), 0.31 g/L NH₄Cl, 0.13 g/L KCl, and trace vitamins and minerals [17].

Voltage to the MECs was applied by a power source (3645A; Circuit Specialists, Inc., AZ). After each MEC cycle, the reactors were draped, exposed to air for 10 min to inhibit methanogen growth [13], refilled with substrate solution (1 g/L unless noted), and sparged with ultra high purity nitrogen gas for 5 min. The reactors were run in duplicate and maintained in a 30 °C constant temperature room.

2.3. Analysis

Continuous gas production was measured using a respirometer (AER-200; Challenge Technology, Springdale, AR). Gas leaving the respirometer was collected in sampling bags (250 mL capacity, Cali-S bond; Calibrated Instruments Inc., Hawthorne, NY). Gas composition (MEC headspace and gas bags) was analyzed by gas chromatography [18]. Liquid product composition was determined by High Performance Liquid Chromatography (LC-10AD; Shimadzu, Japan) with an Aminex HPX-87H column (Bio-Rad Laboratories, Hercules, CA) and 4 mM H₂SO₄ as the mobile phase (0.5 mL/min, 45 °C). Glycerol, glucose, and diols (2,3-butanediol, 1,3-propanediol, 1,2-propanediol) were detected using a refractive index detector (RID). Organic acids (acetate, butyrate, lactate, propionate, succinate) and alcohols (butanol, ethanol) were detected by both RID and ultraviolet (210 nm) detectors.

2.4. Calculations

A charge balance was used to determine the fate of electrons in the MEC [19]. The number of Coulombs (C) can be calculated as:

\[ C = nbF \]  

(1)

where \( n \) is the number of moles, \( b \) the moles of electrons per mole of substrate calculated from the half-cell reaction (Table 1), and \( F \) is Faraday’s constant (\( F = 96484.3 \text{ C/mol} \)). The total Coulombs at any time (\( C_T \)) are calculated as: 
The use of P-glycerol as a substrate in electrohydrogenesis resulted in a high volume of gas (1428 ± 85 mL/g-COD) with a consistent composition at an applied voltage of 0.9 V over 5 consecutive batch cycles (Fig. 1A). Gas production with B-glycerol (444 ± 103 mL/g-COD) was lower than that with P-glycerol. When glucose was used, gas production (1283 ± 42 mL/g-COD) was similar to that achieved with P-glycerol on the basis of COD. The use of a lower applied voltage (0.5 V) produced lower and more variable amounts of gas over successive cycles for both glucose and glycerol (1060 ± 157 mL/g-COD P-glycerol, 530 ± 144 mL/g-COD B-glycerol, 1040 ± 408 mL/g-COD glucose) (Fig. 1B). Similar gas production rates observed here using glucose and glycerol with MECs did not occur during anaerobic fermentation with these two substrates as gas production was two times higher for glucose (296 ± 20 mL/g-COD) than for glycerol (133 ± 18 mL/g-COD) fermentation [5].

The gas was consistently composed of more hydrogen than carbon dioxide or methane at 0.9 V than at 0.5 V. Using P-glycerol, the gas was 88% H$_2$ (994 mL-H$_2$) at 0.9 V, compared to 80% H$_2$ (766 mL-H$_2$) at 0.5 V (Fig. 2, Table 2). This effect of applied voltage on hydrogen gas production was not as substantial with B-glycerol, as the percent of hydrogen gas composition was only slightly higher at 0.9 V (87% H$_2$) than at 0.5 V (82% H$_2$). Similarly, the gas was relatively enriched with hydrogen using glucose at 0.9 V (87%) compared to 0.5 V (81%).

### Table 1 – Half-cell reactions and number of moles of electrons per mole of substrate.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Reaction</th>
<th>b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycerol</td>
<td>$\text{C}_3\text{H}_6\text{O}_3 + 3\text{H}_2\text{O} \rightarrow \text{3CO}_2 + 14\text{H}^+ + 14e^-$</td>
<td>14</td>
</tr>
<tr>
<td>Glucose</td>
<td>$\text{C}_6\text{H}_12\text{O}_6 + 6\text{H}_2\text{O} \rightarrow 6\text{CO}_2 + 24\text{H}^+ + 24e^-$</td>
<td>24</td>
</tr>
<tr>
<td>Acetate</td>
<td>$\text{C}_2\text{H}_4\text{O}_2 + 2\text{H}_2\text{O} \rightarrow 2\text{CO}_2 + 8\text{H}^+ + 8e^-$</td>
<td>8</td>
</tr>
<tr>
<td>Lactate</td>
<td>$\text{C}_3\text{H}_6\text{O}_3 + 3\text{H}_2\text{O} \rightarrow \text{3CO}_2 + 12\text{H}^+ + 12e^-$</td>
<td>12</td>
</tr>
<tr>
<td>Formate</td>
<td>$\text{CH}_2\text{O} \rightarrow \text{CO}_2 + 2\text{H}^+ + 2e^-$</td>
<td>2</td>
</tr>
<tr>
<td>1,3-Propanediol</td>
<td>$\text{C}_3\text{H}_6\text{O}_2 + 4\text{H}_2\text{O} \rightarrow \text{3CO}_2 + 16\text{H}^+ + 16e^-$</td>
<td>16</td>
</tr>
<tr>
<td>Propionate</td>
<td>$\text{C}_3\text{H}_6\text{O}_2 + 4\text{H}_2\text{O} \rightarrow \text{3CO}_2 + 14\text{H}^+ + 14e^-$</td>
<td>14</td>
</tr>
</tbody>
</table>

where $C_t$ are the Coulombs from the measured intermediates (equation (1), Table 1), $C_s$ are the Coulombs calculated from the substrate left in the medium, $C_r$ are the Coulombs recovered from the current produced and $C_{c}$ are the remaining Coulombs lost to non-measured products such as biomass or extracellular polymers. The total Coulombs recovered from current ($C_r$) are calculated by integrating the current over the desired time ($T$) [20]:

$$C_r = \frac{\sum_{i=1}^n V_i}{R}$$

where $V$ is the measured voltage ($V$), $t$ is the time interval (1200 s) and $R$ is the applied resistance (10 $\Omega$).

The performance of the reactors was evaluated using equations given in Logan et al. [6] unless otherwise noted. Coulombic efficiency (CE) (%) was calculated as the percentage of total Coulombs recovered as current to original Coulombs in the substrate. The cathodic hydrogen recovery ($\text{rec}_{\text{cat}}$) (%) was the percentage of electrons that were recovered as hydrogen gas from the total number of electrons that reached the cathode. The volumetric current density ($i_v$) (A/m$^3$) was the average of the maximum current production over a 4-h period divided by the liquid volume. The maximum volumetric hydrogen production rate (Q) (m$^3$ H$_2$/m$^3$ d) was proportional to the current produced and the gas rate per volume of reactor. The hydrogen gas composition (H$_2$%) was calculated by using the gas bag approach calculation ($V_{\text{H}_2}$) (mL) by Logan et al. [6] and dividing it by the total gas produced (mL). The hydrogen yield ($Y_{\text{H}_2}$) (mol-H$_2$/mol-substrate) was the moles of hydrogen produced divided by the moles of substrate consumed. This is the only value adjusted for glycerol content (69.5%) of B-glycerol. The energy efficiency relative to electrical input ($\eta_{\text{el}}$) (%) was the ratio of energy content of hydrogen produced to the electrical energy added. The overall energy recovery based on both electric and substrate inputs ($\eta_{\text{el,sub}}$) (%) takes into account both the electrical input and the heat of combustion of the substrate (glycerol $\Delta H = 1655.4$ kJ/mol, glucose $\Delta H = 2802.7$ kJ/mol) [21].

### 3. Results

#### 3.1 Volumetric gas production and composition

The use of P-glycerol as a substrate in electrohydrogenesis resulted in a high volume of gas (1428 ± 85 mL/g-COD) with...
3.2. Hydrogen yields

Hydrogen yields for P-glycerol at applied 0.9 V reached 3.9 mol-H2/mol-glycerol [Fig. 3]. This is 56% of the maximum possible yield by oxidation (7 mol-H2/mol-glycerol), and above the yield that could be achieved by fermentation to acetate (3 mol-H2/mol-glycerol). Hydrogen yields for B-glycerol were 1.8 mol-H2/mol-glycerol, which is 36% of the theoretical maximum by oxidation, and 85% of that possible by fermentation. Higher hydrogen yields of 7.2 mol-H2/mol-glucose were obtained using glucose, which is 59% of the theoretical maximum by oxidation (12 mol-H2/mol-glucose). Hydrogen yields for the pure substrates were reduced at the lower applied voltage (0.5 V), with 3.1 mol-H2/mol-glycerol (P-glycerol), 2.3 mol-H2/mol-glycerol (B-glycerol), and 6.4 mol-H2/mol-glucose.

3.3. Hydrogen gas production rates

The hydrogen production rate for P-glycerol (1 g/L) at 0.9 V (Q = 2.0 m3/m3 d) was double that obtained at 0.5 V (Q = 0.8 m3/m3 d) (Table 2). Increasing the voltage improved performance of the MEC with P-glycerol in terms of Coulombic efficiency (CE = 99–104%), cathodic recovery (r_{cat} = 64–79%), current (I_{v} = 116–221 A/m3) and total energy efficiency (ν_{E,S} = 47–51%). However, the increased voltage resulted in a decrease in energy efficiency based on electrical input (ν_{E} = 198–139%).

Performance of the MEC with P-glycerol was better than that with B-glycerol. For example, hydrogen production rate (Q) was five times lower for B-glycerol (0.41 m3/m3 d) than P-glycerol (2.0 m3/m3 d) at 0.9 V. Hydrogen production was examined over a range of applied voltages using B-glycerol. Production rates and other MEC performance parameters generally showed a favorable increase with the applied voltage, although at 0.9 V there was a slight decrease in hydrogen production rate (range H2 = 79–87%, Q = 0.14–0.55 m3/m3 d; ν_{E,S} = 28–37% (Table 2, Fig. 4). Energy efficiency based on electrical input (ν_{E}) was optimum at 0.6 V (182%). At 0.3 V, the applied voltage was too small to produce any significant hydrogen (Q = 0.0 m3/m3 d). This sharp drop in performance at a low applied voltage was also observed at 0.2 V in MEC tests by others using acetate [13], where Q = 0.5 m3/m3 d at an applied voltage of 0.3 V, but no gas production occurred at 0.2 V.

3.4. Charge balance analysis

P-glycerol was completely consumed 6 h after the start of the fed batch cycle, with all intermediates consumed within 36 h (Fig. 5A). The main intermediate was 1,3-propanediol (PD), with lesser amounts of acetate and propionate. While PD and acetate were produced almost immediately, propionate formation had a lag time of 4 h. The total Coulombs accounted for by the intermediates reached a peak at 8 h and steadily decreased until the end of the cycle (Fig. 5B). After 8 h, 78% of the Coulombs could be accounted for by intermediates (56%) and by current (22%). Coulomb accountability increased to 100% after 16 h and remained constant for the duration of the cycle.

Glucose was completely removed less than 5 h after the start of the MEC cycle and all intermediates were consumed within

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Table 2 – MEC results for glucose or glycerol.

<table>
<thead>
<tr>
<th>Substrate type</th>
<th>Conc g/L</th>
<th>Applied V V</th>
<th>CE %</th>
<th>r_{P,E,cat} %</th>
<th>I_{v} A/m3</th>
<th>Q m3/m3 d</th>
<th>H2 %</th>
<th>ν_{E} %</th>
<th>ν_{E,S} %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>1</td>
<td>0.5</td>
<td>127 ± 23</td>
<td>51 ± 4</td>
<td>115 ± 4</td>
<td>0.83 ± 0.18</td>
<td>81 ± 5</td>
<td>159 ± 12</td>
<td>50 ± 10</td>
</tr>
<tr>
<td>Glucose</td>
<td>1</td>
<td>0.9</td>
<td>105 ± 10</td>
<td>88 ± 5</td>
<td>182 ± 31</td>
<td>1.87 ± 0.30</td>
<td>87 ± 2</td>
<td>152 ± 8</td>
<td>62 ± 4</td>
</tr>
<tr>
<td>P-Glycerol</td>
<td>1</td>
<td>0.5</td>
<td>99 ± 10</td>
<td>64 ± 15</td>
<td>116 ± 5</td>
<td>0.80 ± 0.08</td>
<td>80 ± 0</td>
<td>198 ± 48</td>
<td>47 ± 7</td>
</tr>
<tr>
<td>P-Glycerol</td>
<td>1</td>
<td>0.9</td>
<td>104 ± 7</td>
<td>79 ± 18</td>
<td>221 ± 12</td>
<td>2.01 ± 0.41</td>
<td>88 ± 2</td>
<td>139 ± 31</td>
<td>51 ± 10</td>
</tr>
<tr>
<td>P-Glycerol</td>
<td>2</td>
<td>0.5</td>
<td>43 ± 1</td>
<td>18 ± 3</td>
<td>116 ± 2</td>
<td>0.10 ± 0.02</td>
<td>31 ± 1</td>
<td>58 ± 10</td>
<td>7 ± 1</td>
</tr>
<tr>
<td>P-Glycerol</td>
<td>3</td>
<td>0.5</td>
<td>49 ± 5</td>
<td>1 ± 1</td>
<td>136 ± 45</td>
<td>0.01 ± 0.01</td>
<td>3 ± 2</td>
<td>4 ± 2</td>
<td>1 ± 0</td>
</tr>
<tr>
<td>B-Glycerol</td>
<td>1</td>
<td>0.3</td>
<td>37 ± 5</td>
<td>1 ± 0</td>
<td>15 ± 3</td>
<td>0.00 ± 0.00</td>
<td>2 ± 0</td>
<td>3 ± 0</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>B-Glycerol</td>
<td>1</td>
<td>0.5</td>
<td>84 ± 11</td>
<td>45 ± 15</td>
<td>35 ± 8</td>
<td>0.14 ± 0.06</td>
<td>82 ± 2</td>
<td>136 ± 44</td>
<td>28 ± 6</td>
</tr>
<tr>
<td>B-Glycerol</td>
<td>1</td>
<td>0.6</td>
<td>65 ± 8</td>
<td>72 ± 19</td>
<td>59 ± 10</td>
<td>0.30 ± 0.01</td>
<td>79 ± 1</td>
<td>182 ± 47</td>
<td>36 ± 6</td>
</tr>
<tr>
<td>B-Glycerol</td>
<td>1</td>
<td>0.8</td>
<td>103 ± 11</td>
<td>52 ± 15</td>
<td>87 ± 11</td>
<td>0.55 ± 0.28</td>
<td>78 ± 13</td>
<td>99 ± 29</td>
<td>35 ± 12</td>
</tr>
<tr>
<td>B-Glycerol</td>
<td>1</td>
<td>0.9</td>
<td>91 ± 10</td>
<td>65 ± 14</td>
<td>63 ± 14</td>
<td>0.41 ± 0.13</td>
<td>87 ± 4</td>
<td>107 ± 25</td>
<td>37 ± 7</td>
</tr>
</tbody>
</table>
30 h (Fig. 6A). Glucose electrohydrogenesis resulted in the rapid formation of lactate, formate, and acetate, with propionate observed only after 4 h. After 8 h, only 55% of the Coulombs were accounted for by current production (25%) and intermediates (19%) (Fig. 6B) compared to 56% for P-glycerol. By the end of the experiment, the recovery of Coulombs was 109%.

3.5. Effect of organic loading

The effect of organic loading was examined by increasing the initial P-glycerol concentration (1, 2 and 3 g/L or 10.9 mM, 21.8 mM and 32.7 mM, respectively) at the lower applied voltage of 0.5 V (Table 2). The performance of the reactors decreased with increased organic loading in terms of all operational parameters (CE = 104–49%, \( \eta_{\text{H}_2, \text{cat}} = 79–1\% \), \( \eta_e = 139–4\% \), \( \eta_{\text{E+S}} = 51–1\% \), \( I_v = 221–136 \, \text{A/m}^3 \), \( Q = 2–0.01 \, \text{m}^3/\text{m}^3 \, \text{d} \) and \( \text{H}_2 \) content = 88–3%). Cycle times (36–48 h) and methane composition (7–80%) also increased with increasing glycerol concentration (1–3 g/L) (Fig. 7).

The impact of the higher organic loading on performance can be better understood through the analysis of intermediates over time (Fig. 8A). At an initial glycerol concentration of 3 g/L, P-glycerol was consumed during the same time span as the lower glycerol concentration of 1 g/L (within 6 h) and formed the same fermentation products (acetate, propionate and 1,3-propanediol) (Fig. 5A). However, 4.6 mM of propionate remained in solution at the end of a cycle, accounting for 20% of the total Coulombs added (Fig. 8B). At the higher P-glycerol concentration of 3 g/L, 49% of the Coulombs were unaccounted for during the cycle (between 20 and 30 h) (Fig. 8B), compared to only 22% at 1 g/L (8 h). The intermediates accounted for 22% and the current accounted for 29% of the Coulombs recovered. The maximum Coulombs accounted at the end of the cycle were 68% at 3 g/L (48% from current, 20% from propionate), compared to 100% at 1 g/L (Fig. 5B).

4. Discussion

4.1. MEC performance in comparison to other studies

The MECs with P-glycerol produced an overall energy efficiency of \( \eta_{\text{E+S}} = 51 \pm 10\% \), a hydrogen production rate of
$Q = 2.0 \pm 0.4 \text{ m}^3/\text{m}^3 \text{ d}$, with a hydrogen yield of $3.9 \text{ mol-H}_2/\text{mol-glycerol}$ at applied $0.9 \text{ V}$. The use of a lower applied voltage ($0.5 \text{ V}$) reduced performance ($\eta_{E+S} = 47 \pm 7\%$, $Q = 0.8 \pm 0.1 \text{ m}^3/\text{m}^3 \text{ d}$, with a hydrogen yield of $3.2 \text{ mol-H}_2/\text{mol-glycerol}$) and there was greater variability between cycles. These results are up to five times higher than those achieved in a previous study with glycerol using a two-chamber MEC and an exogenous mediator ($0.77 \text{ mol-H}_2/\text{mol-glycerol}$) at a lower applied voltage of $0.2 \text{ V}$ [12].

The performance of the MECs with glucose was better than that achieved with glycerol, in terms of energy efficiency, hydrogen production rates ($\eta_{E+S} = 62\%$, $Q = 1.87 \text{ m}^3/\text{m}^3 \text{ d}$), and hydrogen yield ($7.2 \text{ mol-H}_2/\text{mol-glucose}$) (applied $0.9 \text{ V}$). Results with glucose at an applied voltage of $0.9 \text{ V}$ were comparable to those in a previous two-chamber MEC study at a lower applied voltage of $0.6 \text{ V}$ in terms of these same parameters ($\eta_{E+S} = 64\%$, $Q = 1.23 \text{ m}^3/\text{m}^3 \text{ d}$, and hydrogen yield of $8.6 \text{ mol-H}_2/\text{mol-glucose}$) [16]. Both of these results with glucose are better than those reported in another two-chamber continuous-flow system which had a hydrogen production rate of $Q = 0.58 \text{ m}^3/\text{m}^3 \text{ d}$ at an applied voltage of $1.2 \text{ V}$ [22].

### 4.2. Product formation in MECs

Both glycerol and glucose are fermentable substrates, and it was observed that intermediates rapidly accumulated in solution at the start of the fed batch cycles. This observation in MEC tests of intermediate accumulation was similar to that...
found in MFC tests where it was observed that 84% of the starting concentration of xylose was depleted within 10 h with intermediates formed and consumed over the rest of the 60 h cycle [19].

One of the main intermediates formed during MEC operation with glucose was propionate. Propionate was produced after a lag time of about 4 h, with a peak in concentration 16 h after glucose was completely consumed (5 h). This lag time and observation of continued intermediate formation after the primary substrate was depleted were not observed in MFC tests with xylose [19] where all the intermediates were formed at their maximum concentrations by the time xylose was depleted. This suggests that the propionate formed did not come directly from glucose or glycerol fermentation, but rather from other intermediates such as lactate [23] or acetate [24]. Propionate could have been produced by the same bacteria that may be involved in current generation, for example by Pelotomaculum thermopropionicum [25]. At an initial concentration of 1 g/L of P-glycerol, propionate reached a maximum concentration of 0.8 mM but was subsequently removed. However, at a higher initial concentration of glycerol (3 g/L P-glycerol), propionate accumulated to a higher concentrations (4.6 mM) and it was not removed by the end of the cycle (48 h). The reason for the lack of propionate removal is not known. Propionate accumulation has been observed in anaerobic digestion, and many theories have been proposed for propionate accumulation, such as hydrogen inhibition [26,27].

There was evidence of substantial carbon storage, especially with glucose as substrate. Current and intermediates did not fully account for all the Coulombs (based on COD removal) during some portions of the total cycle time and electron recovery was 100% or higher at the end of the cycle. Carbon storage occurs when bacteria take up substrate and store it in the form of polymers (commonly poly-β-hydroxyalkanoates and glycogen). These stored materials can subsequently be used as energy sources when needed [28]. Carbon storage has been observed in MFC tests with glucose, acetate and xylose, where carbon storage was observed up to 56% of the Coulombs available [19,28]. Carbon storage was not as prominent in MEC tests here using P-glycerol, where a maximum of only 22% of the Coulombs were unaccounted (after 16 h). This shows that for P-glycerol consumption rates are comparable to production rates (intermediates, biomass and current) without much less intracellular carbon storage compared to other fermentable substrates such as glucose and xylose.

Formation of fermentation products and carbon storage indicate that intermediates were the main chemicals used for subsequent current generation for a large portion of the fed batch cycle as opposed to the starting material. The minimum potentials needed for chemical oxidation of fermentation products is larger than that needed for the original substrate. For example, less energy is theoretically needed to form hydrogen at the cathode ($E_{cat} = -0.414 V$) with glucose ($E_{an} = -0.428 V$, $E_{emt} = 0.014 V$) or glycerol ($E_{an} = -0.403 V$, $E_{emt} = -0.011 V$) than is needed for acetate ($E_{an} = -0.3 V$, $E_{emt} = -0.114 V$) [8]. According to these values, glucose oxidation could result in spontaneous current generation in an MEC, but once the intermediates are formed, a spontaneous reaction leading to hydrogen production at the cathode is no longer possible [8].

4.3. Electron recycling and methane production

There was evidence for electron recycling during MEC operation, especially with glucose at an applied voltage of 0.5 V as Coulombic recovery exceeded 100%. Electron recycling occurs when the hydrogen produced at the cathode is used at the anode. Many bacteria in mixed anaerobic cultures can use hydrogen as an electron donor to reduce volatile fatty acids [29]. In addition, exoelectrogens such as Sheuanaella oneidensis MR-1 [30] and Geobacter sulfurreducens [31] can use hydrogen as an electron donor.

Methane formation was substantially reduced at 0.9 V compared to 0.5 V for P-glycerol and glucose. In previous studies, higher voltages corresponded to shorter cycle times and reduced methane formation [5,18]. In this study, the increased voltage did not reduce the cycle time (36 h for both voltages), but methane production still decreased by using a higher applied voltage for P-glycerol and glucose. For B-glycerol, there was no significant change in methane production with applied voltage. Methane formation was most likely linked to propionate formation, as propionate is an important intermediate in the anaerobic degradation of organic matter to methane [32]. Methane was likely formed during the conversion of intermediates to propionate, as methane can be the most predominant byproduct in the gas phase when volatile organic acids are converted to other volatile organic acids or alcohols [29].

4.4. Outlook for hydrogen production from B-glycerol

Results in this study demonstrate that the B-glycerol byproduct from biodiesel fuel production can be converted, without purification, into hydrogen at higher production rates and yields than fermentation. The cost of hydrogen produced via electrohydrogenesis due to the electrical energy input with B-glycerol is $1.06/kg H_2 at applied 0.5 V and $1.18/kg H_2 at applied 0.9 V, based on energy demands of 2.32 kWh/m^3 H_2 (0.5 V) and 2.60 kWh/m^3 H_2 (0.9 V) and current wholesale electricity prices of $41/MWh (www.eia.doe.gov). These costs are 2.5 times less than that needed for hydrogen produced by water electrolysis ($2.55/kg H_2 based on energy requirements of 5.6 kWh/m^3 H_2) [13]. Hydrogen produced by an MEC based on electrical energy would therefore cost one third of that currently charged for pure hydrogen gas ($3–4/kg H_2; Sigma Aldrich, St. Louis, MO).

The main issue remaining with using B-glycerol as a substrate in an MEC is the adverse effect of components other than the glycerol on treatability. B-glycerol is a mixture of glycerol, sodium sulfate, soaps, methanol, and water [33]. Proteins may also be present in B-glycerol depending on the feedstock and the production method [33], although they would be expected to contribute to current production. Single proteins, such as bovine serum albumin, as well a complex mixtures of proteins (peptone and a meat packing waste-water) have been used as substrates in MFCs [34].

The lower MEC performance using B-glycerol compared to P-glycerol is likely mainly due to methanol. Methanol can be
toxic to microorganisms [35], and in tests with methanol in an MFC, which is a system very similar to an MEC, there was no sustained power generation [36]. In fermentation tests with Enterobacter aerogenes, it was also found that there was less hydrogen production with B-glycerol (0.7 mol-H₂/mol-glycerol) than P-glycerol (0.89 mol-H₂/mol-glycerol) [2]. Methanol can be removed by evaporation. A detailed economic analysis is needed to determine if the increased cost of methanol removal would be justified by the increased hydrogen yield.

The effect of triglycerides on hydrogen production in an MEC is not clear. Triglycerides have not been used in MFC or MEC systems, but it is likely that they could be used as a substrate for power generation or just degraded via fermentation. Pseudomonas aeruginosa, which has been shown to produce current in an MFC using self-produced mediators [37], can ferment triglycerides at higher rates than glycerol [38]. Triglycerides can also be converted into soaps, and this could negatively influence bacterial growth. Anionic and cationic surfactants, for example, can restrain or retard the growth of P. aeruginosa [39]. Soaps have been found to have a negative effect on docosahexaenoic acid production by algae via fermentation from B-glycerol [40]. Soap removal can be difficult, but a common method for removal is by precipitation through pH adjustments (acidic conditions) [40].

It is unlikely that sodium sulfate in B-glycerol was a factor in reduced hydrogen production. Sodium sulfate is produced from the neutralization of sodium hydroxide with sulfuric acid during the esterification process in biodiesel production. Sodium is required by some but not all organisms, depending on their natural habitat [41]. Sulfate is a source of sulfur for most organisms and is used by sulfate-reducing bacteria, which include Geobacter metallireducens and G. sulfurreducens, well-known exoelectrogens [37]. Salts at high concentrations may inhibit cell growth. Reduced hydrogen production by fermentation was observed with E. aerogenes when 1% sodium chloride was added to 10 g/L B-glycerol (~200 mM NaCl plus salts in B-glycerol) but not when it was added to P-glycerol (171 mM NaCl) [2].

5. Conclusions

High hydrogen yields were obtained from glycerol and glucose in single-chamber membraneless MEC reactors. A higher applied voltage (0.9 V) than that typically used for acetate (0.5 V) was needed for consistent operation and methane reduction. The fermentable substrates were consumed rapidly and fermentation products were formed depending on the substrate. 1,3-Propanediol was the main intermediate during glycerol electrohydrogenesis, while acetate and propionate were the main products during glucose electrohydrogenesis. All intermediates were consumed by the end of an MEC cycle at 1 g/L, but not at 3 g/L where 4.6 mM propionate remained at the end of the cycle. Electron recycling and cell carbon storage occurred during glucose electrohydrogenesis, and to a smaller extent during glycerol electrohydrogenesis. B-glycerol produced higher hydrogen yields in MEC than anaerobic fermentation, but less than those with P-glycerol, probably due to the presence of methanol and soaps in the mixture and complexity of the substrate.

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