Hydrogen and methane production from swine wastewater using microbial electrolysis cells

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A B S T R A C T

The production of a useful and valuable product during swine wastewater treatment, such as hydrogen gas, could help to lower treatment costs. Hydrogen can theoretically be produced from wastewater by electrohydrogenesis in a microbial electrolysis cell (MEC) or by fermentation. Using a single-chamber MEC with a graphite-fiber brush anode, hydrogen gas was generated at 0.9–1.0 m³ m⁻³ day⁻¹ H₂ using a full-strength or diluted swine wastewater. COD removals ranged from 8 to 29% in 20-h tests, and from 69 to 75% in longer tests (184 h) using full-strength wastewater. The gas produced was up to 77/₁₀₁₁₁₁% hydrogen, with overall recoveries of up to 28 ± 6% of the COD in the wastewater as hydrogen gas. Methane was also produced at a maximum of 13 ± 4% of total gas volume. The efficiency of hydrogen production, based on the electrical energy needed (but excluding the energy in the wastewater) compared to the energy of the hydrogen gas produced, was as high as 190/₁₀₃₉₃₉% in 42-h batch tests with undiluted wastewater, but was lower in longer batch tests of 184 h (91 ± 6%). Hydrogen gas could not be recovered in fermentation tests using wastewater with a heat-treated inoculum. Hydrogen production was shown to be possible by fermentation when the wastewater was sterilized, but this process would not be practical or energy efficient. We therefore conclude from these tests that MECs are an effective method for hydrogen recovery from swine wastewater treatment, although the process needs to be further evaluated for reducing methane production, increasing the efficiency of converting the organic matter into current, and increasing recovery of hydrogen gas produced at the cathode.

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1. Introduction

Considerable amounts of animal wastewater are generated each year that require extensive treatment. In the US alone there are 64 million hogs and the amount of animals being used in food production is increasing (National Agricultural Statistics Service, 2007). Conventional methods of treating animal wastewaters include anaerobic lagoons, constructed...
Energy can be extracted from wastewater during treatment, providing products that can help offset treatment costs. Microbial fuel cells (MFCs) have been examined as a method for generating electricity while simultaneously treating wastewater (Logan, 2005). In these systems, bacteria oxidize organic matter and release electrons to an anode, which then flow to the cathode and combine with oxygen and protons to form water. Swine wastewater was successfully treated using MFCs (Min et al., 2005), and it was recently shown that MFCs could also be used to remove odors (Kim et al., 2008). A more conventional approach to swine wastewater treatment is anaerobic digestion, in which organic matter is broken down by bacteria, releasing volatile fatty acids and hydrogen gas. These intermediate products are used by methanogens to produce methane. Hydrogen gas, however, contains more energy (on a mass basis) and is therefore more valuable than methane. Hydrogen has been successfully produced by fermentation using food processing wastewaters, municipal wastewater sludge filtrate, and paper hydrolysates (Van Ginkel et al., 2005; Wang et al., 2004), as well as from solids such as wheat starch, bean curd waste, wheat and rice bran, and municipal solid wastes (Kalia et al., 1994; Lay et al., 1999; Noike and Mizuno, 2000; Okamoto et al., 2000). Recently, hydrogen from domestic sewage sludge fermentation was reported (Massanet-Nicolau et al., 2008), but both enzymatic and heat pretreatments were necessary for hydrogen production. While recovering hydrogen gas from swine wastewater may allow for a more cost-effective treatment process, high hydrogen gas yields have not yet been achieved using swine wastewater by a fermentation process.

Hydrogen gas can also be produced from biomass using electrohydrogenesis (Cheng and Logan, 2007), in a device called a microbial electrolysis cell (MEC; Logan and Grot, 2005; Liu et al., 2005). The MEC is a modified MFC in which the cathode is completely anoxic, and a voltage is added to that produced by the bacteria to allow for hydrogen evolution. At the cathode, electrons combine with protons to form hydrogen via the hydrogen evolution reaction (HER): \( 2H^+ + 2e^- \rightarrow H_2 \) (g). Bacteria at the anode consume organic matter and produce a voltage of approximately \(-0.3\) V, while the HER requires \(-0.41\) V, requiring a theoretical input of \(0.11\) V. In practice, a higher voltage input of \(0.25\)–\(0.8\) V is required for the HER to occur in an MEC (Logan, 2008). It has been shown that three times as much energy can be recovered in the hydrogen gas than is added as electrical energy using the process with acetate and several other volatile fatty acids (Cheng and Logan, 2007).

The purpose of this study was to examine the feasibility of MECs for fermentation for producing hydrogen gas from swine wastewater. In both methods, the main barrier to hydrogen recovery is hydrogen consumption by methanogens. There are very few studies on using MECs for hydrogen generation, and only one using wastewater. Current was produced in an MEC using domestic wastewater, but the low strength of the wastewater required the use of relatively high added voltages, and the reactor had high internal resistance leading to low hydrogen recoveries (Ditzig et al., 2007). A new single-chamber MEC reactor was recently designed (Call and Logan, 2008) that has a lower internal resistance than the MEC used by Ditzig et al. (2007), and it produced much higher hydrogen gas flow rates from acetate than in other MEC studies (Rozendal et al., 2006, 2007). We therefore examined hydrogen production using this MEC reactor with swine wastewater to see if we could achieve reasonable hydrogen recoveries. We compared this approach to a more conventional fermentation-based approach using a heat-treated inoculum to select for non-methanogenic microorganisms, and determined the upper limit for the efficiency of a fermentation-based approach by completely sterilizing the wastewater. We demonstrate here that while fermentation of swine wastewater does not produce hydrogen without energy-intensive pretreatments, electrohydrogenesis in an MEC can easily achieve high hydrogen recoveries even in a single-chamber reactor.

### 2. Materials and methods

#### 2.1. Swine wastewater

Swine wastewater was collected from the slurry pits of the swine farm located at the Pennsylvania State University in University Park, PA and stored at 4 °C for <30 days. The wastewater had a chemical oxygen demand (COD) of approximately 12,000–17,000 mg/L.

#### 2.2. Microbial electrolysis cell

In preparation for MEC operation, MFCs were constructed of lexan, with an air cathode with a platinum catalyst (Logan et al., 2007). Graphite-fiber brush anodes were enriched with exoelectrogenic bacteria using diluted swine wastewater (COD = 2000 mg/L) in duplicate, single-chamber MFCs having a cylindrical chamber 4-cm long by 3-cm in diameter (empty volume = 28 mL). After acclimation, the MFCs consistently produced a maximum voltage of approximately 550 mV. After at least three consistent cycles at this maximum voltage, the reactors were converted to operate as MECs as previously described (Call and Logan, 2008) by covering the air cathode with a plate to exclude air and eliminating the oxygen reduction at the cathode. Gas was collected from an anaerobic culture tube glued to fit on top of a 1.6-cm opening on the top of the reactor. MECs were exposed to air for 30–60 min after each cycle to reduce methane production by oxygen-sensitive methanogens that could be present in the biofilm. Gas production from the reactor was measured using a respirometer (AER-200, Challenge Environmental). A power source (3645A; Circuit Specialists, Inc.) was connected to the circuit to add voltage, and a multimeter (2700; Keithley Instruments, Inc.) was used to monitor the voltage across an external resistor \(R_{ex} = 10\Omega\) to calculate current. For all experiments, 0.5 V was applied from the external power source to the reactor. All tests were run at 30 °C in a constant temperature...
room. All tests used non-diluted (ND) wastewater without amendments, except (as noted) in some tests the wastewater was diluted (D) to a COD of 2000 mg/L and buffered (50 mM phosphate buffer, pH = 7) with trace minerals (per liter: 15 mg NTA, 30 mg MgSO₄·7H₂O, 5 mg MnSO₄·H₂O, 10 mg NaCl, 1 mg FeSO₄·7H₂O, 1 mg CoCl₂·6H₂O, 1 mg CaCl₂, 1 mg ZnSO₄·7H₂O, 0.1 mg CuSO₄·5H₂O, 0.1 mg Al₂(SO₄)₃·12H₂O, 0.1 mg H₂BO₃, 0.1 mg Na₂MoO₄·2H₂O) and trace vitamins (per liter: 2 mg Biotin, 2.0 mg Folic acid, 10 mg Pyridoxine hydrochloride, 5 mg Thiamine HCl, 5.0 mg Riboflavin, 5.0 mg Nicotinic acid, 5.0 mg Calcium D-(-)-pantothenate, 0.1 mg Vitamin B12, 5 mg p-Aminobenzoic acid, 5.0 mg Thioctic acid) (Balch et al., 1979). The non-diluted wastewater had a pH of around 6.8; ammonia was not measured for this experiment, but wastewater from the Penn State swine farm typically has ammonia concentrations of approximately 2000 mg-N/L.

Reactors were operated in fed-batch mode for two different lengths of time, referred to as “long” (L) or “short” (S) batch cycles. A long batch cycle was conducted until the complete gas production cycle ended, as indicated by zero gas production rate for one hour or more. For short batch cycles, the test was discontinued once the gas production rate had reached a peak.

2.3. Measurements and chemical analyses

Total COD analysis of the solution was performed at the beginning and end of each batch cycle (method 8000; HACH COD system, HACH Company, Loveland, CO; Jirka and Carter, 1975). Sugar was analyzed using the phenol-sulfuric acid method for reducing sugars (Dubois et al., 1956), with sample filtration through a 0.2-μm pore diameter membrane filter for dissolved sugar. Total ammonia (ammonia and ammonium ion) was analyzed using an ATI Orion Model 720A Benchtop pH/ISE meter and an ammonia probe (ATI Orion, Boston, MA). A pH probe and meter (Fisher Scientific accumet® model 10 and VWR SympHony) were used for pH measurements. Redox potential in the continuous flow fermentation experiments was measured with a redox probe (Combination Red/Ox probe Pt4805-DXX-58/225, Mettler-Toledo, Columbus, OH). In the fermentation tests, the concentrations of solvents, alcohols, and organic acids (acetone, ethanol, propanol, butanol, acetate, propionate, and butyrate) in the liquid phase were measured by gas chromatography (Varian Star 3400) with injector and flame ionization detector temperatures of 250°C. After each batch cycle, gas from a gas bag and the reactor headspace was sampled using a gas-tight syringe (100 or 200 μL injection volume) and analyzed by gas chromatography for hydrogen, methane, carbon dioxide, and nitrogen gas (GC; Models 310 & 8610B, SRI Instruments, Torrence, CA) (Call and Logan, 2008).

2.4. Calculations

The cathodic hydrogen recovery efficiency, defined as the fraction of electrons reaching the cathode that are recovered as hydrogen gas, is calculated as

\[ r_{H_2,\text{cat}} = \frac{n_{H_2}}{n_{H_2,\text{cat}}} \]  (1)

where \( n_{H_2} \) is the number of moles of hydrogen recovered \( (n_{H_2} = 4.02 \times 10^{-5} \nu_{H_2}) \), and \( n_{H_2,\text{cat}} \) is the number of moles of hydrogen that can theoretically be produced from the current, calculated as

\[ n_{H_2,\text{cat}} = \frac{\sum I \Delta t}{2F} \]  (2)

where 2 is used to convert moles of electrons to moles of hydrogen. Total ammonia (ammonia and ammonium ion) was analyzed using an ATI Orion Model 720A Benchtop pH/ISE meter and an ammonia probe (ATI Orion, Boston, MA). In the fermentation tests, the concentrations of solvents, alcohols, and organic acids (acetone, ethanol, propanol, butanol, acetate, propionate, and butyrate) in the liquid phase were measured by gas chromatography (Varian Star 3400) with injector and flame ionization detector temperatures of 250°C. After each batch cycle, gas from a gas bag and the reactor headspace was sampled using a gas-tight syringe (100 or 200 μL injection volume) and analyzed by gas chromatography for hydrogen, methane, carbon dioxide, and nitrogen gas (GC; Models 310 & 8610B, SRI Instruments, Torrence, CA) (Call and Logan, 2008).

The overall hydrogen recovery, \( r_{H_2,\text{COD}} \), which is defined as the ratio of the hydrogen recovered to the maximum possible hydrogen recovery based on the organic matter oxidized in the wastewater on the basis of COD, is

\[ r_{H_2,\text{COD}} = \frac{n_{H_2}}{n_{H_2,\text{COD}}} \]  (3)

where \( n_{H_2,\text{COD}} = \Delta \text{COD}_{\text{H}_2} b_\text{H}_2/\text{S}, \) \( \Delta \text{COD} \) is the COD (mg/L) consumed during the batch cycle, \( v_\text{b} \) is the volume of the liquid, and \( b_\text{H}_2/\text{S} \) is a conversion factor based on stoichiometric conversion of electrons in COD to hydrogen gas equaling 1 mol H₂ per 16 g O₂.

The Coulombic efficiency (CE), or the fraction of electrons obtained from the consumption of COD that are available for hydrogen production at the cathode, is

\[ \text{CE} = \frac{n_{H_2,\text{cat}}}{n_{H_2,\text{COD}}} = \frac{r_{H_2,\text{COD}}}{r_{H_2,\text{cat}}} \]  (4)

The energy recovery, \( \eta_W \), is the ratio of the energy content of the hydrogen recovered compared to the electrical energy input required for the cathodic reaction. In terms of the number of moles of hydrogen recovered compared to the energy consumed by the power source converted into equivalent moles of hydrogen, energy recovery is calculated as

\[ \eta_W = \frac{n_{H_2}}{n_{H_2,\text{PS}}} \]  (5)

A small external resistor (10 Ω) is used to measure current, so the energy added by the power source, \( W_{\text{PS}} \), is corrected for losses across the external resistor as previously described (Cheng and Logan, 2007; Call and Logan, 2008; Logan et al., 2008) by the following equation:

\[ W_{\text{PS}} = \sum_{1}^{n} I E_{\text{PS}} \Delta t - \sum_{1}^{n} I^2 R_{\text{ex}} \Delta t \]  (6)

The equivalent number of moles of hydrogen is then calculated as

\[ n_{H_2,\text{PS}} = \frac{W_{\text{PS}}}{\Delta H_{\text{H}_2}} \]  (7)

where \( E_{\text{PS}} = 0.5 \) V is the voltage applied using the power source, \( \Delta t (s) \) is the time increment for \( n \) data points measured during a batch cycle, and \( \Delta H_{\text{H}_2} \) is the upper heating value of the heat of combustion of hydrogen gas (285.83 kJ/mol).

Energy added by the power source is normalized by COD removed as \( W_{\text{PS}}/(\Delta \text{COD}_{\text{H}_2}) \). Note that this result is different from a calculation using the applied voltage of the power source (\( E_{\text{app}} \)) and assuming all of the electrons from the COD removed are harvested as current, or \( E_{\text{app}} F b_{\text{H}_2/\text{COD}} \), where the
constant \( b_{\text{COD}} \) is used to convert COD removed to electrons (\( b_{\text{COD}} = 1 \text{ mol e}^-/8 \text{ g COD} \)). For example, at \( E_{\text{app}} = 0.5 \text{ V} \), the maximum energy requirement based on the applied voltage and assuming all COD removed was recovered as current would be 1.67 kWh/kg COD.

The recovery of methane from COD (\( r_{\text{CH}_4,\text{COD}} \)), defined as the ratio of methane produced to the maximum possible methane recovery based on organic matter oxidized, is

\[
r_{\text{CH}_4,\text{COD}} = \frac{n_{\text{CH}_4}}{n_{\text{CH}_4,\text{COD}}}
\]

where \( n_{\text{CH}_4} = \Delta \text{COD} V_{b} b_{\text{CH}_4/S} \) and \( b_{\text{CH}_4/S} = 1 \text{ mol CH}_4/64 \text{ g O}_2 \).

### 2.5. Fermentation experiments

#### 2.5.1. Hydrogen production testing

Batch tests were conducted in glass bottles (299 mL capacity; Wheaton Scientific) using diluted wastewater volumes (200 mL) as previously described (Oh et al., 2003). Swine wastewater was diluted either 2\( \times \) or 8\( \times \) using distilled water and tested at initial pH values of 7.2 (buffered with a 0.02 M phosphate buffer) and 5.8 (buffered with 0.05 M 2-(N-morpholino)ethanesulfonic acid monohydrate [MES; J.T. Baker]), with initial pH adjustments made using HCl or NaOH. Bottles were sparged with nitrogen to remove oxygen and were capped with a rubber stopper and an aluminum seal. All tests were run in duplicate at 30 °C. Biogas production in each test was continuously measured using a respirometric system as previously described (Logan et al., 2002).

Various inocula and sample pretreatment methods were evaluated for their effectiveness in increasing hydrogen production from fermentation. Tests were conducted using: (1) no inoculum; (2) a sludge inoculum consisting of 6.6 g of dewatered sludge collected from the Pennsylvania State University Wastewater Treatment Plant in State College, PA; or (3) a heat-treated (HT) inoculum consisting of 1.0 g dewatered anaerobic sludge (0.2-cm thick) baked for two hours at 104 °C. To assess the maximum hydrogen production possible from the organic matter in the sludge under conditions that theoretically eliminate methanogenic losses of hydrogen, a swine wastewater sample was autoclaved (121 °C, 15 psi) and combined with an HT sludge inoculum at a pH of 5.8. To assess the amount of hydrogen production possible in the presence of indigenous methanogens and other hydrogen-consuming microbes, glucose (1 g) was added to diluted swine wastewater with an HT inoculum (pH = 5.8).

Since methanogens are slow growing and can often be washed out in continuous flow reactors with short hydraulic retention times (HRTs; Rittmann and McCarty, 2001), the possibility of fermentative hydrogen production from swine wastewater was further investigated in continuous culture under optimal conditions to assess the maximum potential for hydrogen production. Wastewater was pumped into a fermentor (New Brunswick BioFlo 110) with an operating volume of 1 L, an HRT of 24 h, and an HT agricultural soil inoculum (Logan et al., 2002). While the microbial composition of this inoculum differs from that used above in the batch tests, previous work has demonstrated equivalent hydrogen production using either inoculum (Oh et al., 2003). The reactor was initially filled with an equal mixture of swine wastewater and distilled water. L-Cysteine (0.5 g/L) was initially added to consume oxygen in the medium, thereby reducing the redox potential, and the solution pH was adjusted to 5.5 with 1 M KOH. The reactor was sparged with nitrogen gas for 1 h and inoculated with the HT agricultural soil (5 g). In an additional test, the wastewater was first sterilized by autoclaving before being introduced to the reactor. In a third test, glucose (5 g/L) was initially added to the reactor to ensure the germination of hydrogen-producing bacteria.

In each case, the reactor was operated in batch mode until the redox potential was reduced to below −200 mV (~3 days, measured against the Argenthal system), and then switched from batch to continuous flow. In continuous flow operation, the reactor was only fed with swine wastewater diluted 2\( \times \) with distilled water with no additional L-Cysteine or glucose. The feed bottle was continuously sparged with nitrogen gas to maintain anaerobic conditions, and the reactor pH was maintained at 5.5 by automated addition of KOH. Gas production was measured daily using a water exclusion apparatus, and hydrogen concentrations in the headspace were measured every 24 h.

#### 2.5.2. Hydrogen consumption testing

To measure the potential for acetogenesis from hydrogen gas, we conducted hydrogen consumption tests using the HT sludge inoculum at four different pHs (5, 6, 7, and 8). In previous tests using glucose, methanogenesis was successfully eliminated by a combination of low pH and heat treatment of the inoculum (Logan et al., 2002). Serum bottles were inoculated with HT sludge (0.5 g), filled with 200 mL of sulfate-free nutrient mineral solution (0.5 M phosphate buffer, 1 g sodium bicarbonate), and the initial pH adjusted with HCl or NaOH. Bottles were flushed with hydrogen gas and capped with a rubber stopper and an aluminum seal. CO\(_2\) (50 mL) was added to the bottles using a syringe, and the pressure was then released using a needle. Abiotic (no inoculum) and biotic (inoculum with nitrogen headspace) controls were also prepared. Hydrogen in the headspace was measured over time.

### 3. Results

#### 3.1. Hydrogen production and wastewater treatment in microbial electrolysis cells

Hydrogen gas was produced in all MEC tests. In the short-cycle (S) batch tests, 17 ± 7% of the COD removed was recovered in hydrogen gas (14 ± 5 mL) using non-diluted (ND) wastewater (20 h cycle time), while 22 ± 4% of the COD was recovered in hydrogen gas (9 ± 3 mL) using the diluted wastewater (16 h cycle time; Fig. 1, Table 1). Using a long-cycle (L) time required for completion of hydrogen production for the diluted (D) sample (42 h) slightly increased conversion of COD removed to hydrogen gas to 28 ± 6%, and the volume of hydrogen gas produced to 15 ± 2 mL. A complete cycle time of the non-diluted sample required 184 h and resulted in a similar conversion efficiency of COD removed to hydrogen of 20 ± 1%, but the volume of hydrogen gas increased to 77 ± 11 mL due to combined high COD concentration and duration of the test.
The energy yield was 190 ± 39% based on the energy content of the hydrogen gas recovered in the tests with the non-diluted wastewater in the short batch cycle (ND-S; Fig. 1). COD was reduced by 19 ± 15% in the ND-S test (Fig. 1), with the best COD reduction of 72 ± 4% achieved in the longer test with non-diluted wastewater (ND-L; Fig. 1). However, the ND-L experiment was the least efficient with respect to energy recovery as hydrogen versus required energy input (ηw = 91 ± 6%), in large part due to the long time of the test (184 h), which resulted in current generation, and therefore power input, approximately 10 times greater than the D-L test (42 h), but a volume of hydrogen only approximately 5.5 times greater. A larger portion of the current was recovered as methane in the longer test (ND-L), which produced nearly 13 times as much methane as the shorter test (D-L). The other tests had energy efficiencies of 179 ± 4% and 165 ± 21% for the D-L and D-S tests respectively, based on the electrical energy input and the hydrogen recovered. Energy input from the power source ranged from 0.47 to 1.09 kWh/kg COD, with an average of 0.8 kWh/kg COD.

There was a large variation in the recovery (as current) of electrons from the COD removed, with CEs ranging from 4% achieved in the longer test with ND-L, which produced nearly 13 times as much methane as the shorter test (D-L). The other tests with cycle times of 16–42 h achieved cathodic hydrogen recoveries of 53–61%.

The hydrogen production rate (Q) was similar in all tests. It was approximately 1.0 m³ m⁻³ day⁻¹ H₂ (total reactor volume) for the long batch cycle time tests, and 0.9 m³ m⁻³ day⁻¹ H₂ for the short batches (Table 1).

The MECs produced methane in addition to hydrogen (Table 1). Hydrogen was 77 ± 5% of the total gas volume for the D-L test, and 74 ± 4% for the D-S test. For the tests using non-diluted wastewater, the percentage of hydrogen in the gas was slightly smaller, with 64 ± 1% for the ND-L test and 58 ± 1% for the ND-S test. The balance in each case was methane and carbon dioxide. Methane recovery from COD (rCH₄/COD) was slightly less than hydrogen recovery from COD for all tests, between 5 ± 2% to 21 ± 7% for rCH₄/COD and from 17 ± 7 to 28 ± 6% for rH₂/COD (Fig. 2).

3.2. Hydrogen production and consumption in fermentation tests

Fermentation tests were conducted under various conditions in order to evaluate the potential for hydrogen production. However, even with the addition of an HT inoculum and conditions that limit hydrogen-consuming reactions (the wastewater was autoclaved and the solution buffered to pH 5.8), hydrogen gas only reached a concentration of 6% in the headspace after 75 h (Fig. 3). After 200 h, the hydrogen gas was completely re-consuming by the culture. The amount of energy needed to autoclave the wastewater (approximately 1–2 kWh/kg COD) would not justify the small amount of hydrogen produced (equivalent to <0.001 kWh/kg COD, data not shown), but this procedure does demonstrate that the upper limit for hydrogen production under optimal conditions by fermentation is quite small. In tests with wastewater (no inoculum amendment, buffered to either pH 5.8 or 7.2), no net hydrogen was produced. There was biogas production (with or without a sludge inoculum), but the gas contained methane and carbon dioxide, and no hydrogen. When the pH was lowered to inhibit methanogenesis (adjusted to 5.8 and to 5.0), gas production decreased but still no hydrogen was produced.

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Batch cycle time (h)</th>
<th>Initial COD (mg/L)</th>
<th>CE (%)</th>
<th>rH₂ cathode (%)</th>
<th>rH₂ COD (%)</th>
<th>rCH₄ COD (%)</th>
<th>Q (m³ m⁻³ day⁻¹ H₂)</th>
<th>Current densities H₂ (A/m³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-S</td>
<td>16</td>
<td>2000</td>
<td>43 ± 2</td>
<td>53 ± 6</td>
<td>23 ± 4</td>
<td>21 ± 2</td>
<td>0.8 ± 0.2</td>
<td>93 ± 22</td>
</tr>
<tr>
<td>ND-S</td>
<td>20</td>
<td>12,825</td>
<td>29 ± 17</td>
<td>61 ± 12</td>
<td>17 ± 7</td>
<td>5 ± 2</td>
<td>0.9 ± 0.2</td>
<td>106 ± 6</td>
</tr>
<tr>
<td>D-L</td>
<td>42</td>
<td>2000</td>
<td>48 ± 9</td>
<td>58 ± 1</td>
<td>28 ± 6</td>
<td>21 ± 7</td>
<td>1 ± 0.1</td>
<td>92 ± 13</td>
</tr>
<tr>
<td>ND-L</td>
<td>184</td>
<td>12,825</td>
<td>70 ± 2</td>
<td>29 ± 2</td>
<td>20 ± 1</td>
<td>14 ± 5</td>
<td>1 ± 0.1</td>
<td>112 ± 25</td>
</tr>
</tbody>
</table>

a Short-cycle = batch ended immediately after peak gas production rate; long-cycle = batch ended when gas production ceased.

b Maximum rate sustained for ≥2 h.
Additional tests were conducted to see if other factors were interfering with hydrogen production. Glucose was added to wastewater to ensure germination of hydrogen-producing bacteria, the wastewater was diluted either 2 or 8 to reduce the concentration of methanogens and ammonia, and a low pH was used to restrict methanogenesis (pH = 5.8). In tests with 2 or 8 diluted and autoclaved wastewater, HT inoculum, and glucose, the biogas produced was approximately 50% hydrogen (Fig. 4). Without the HT inoculum or autoclaving, the 2 diluted wastewater with glucose produced biogas with approximately 23% hydrogen gas over 70 h. When 2 diluted and autoclaved wastewater with HT inoculum was tested without any glucose addition, the hydrogen in the biogas was <10%. The molar hydrogen yields were 1–1.2 mol H₂/mol glucose for the HT sludge alone (not plotted in Fig. 4), 1.89 for the 2 diluted wastewater with HT sludge, and 0.59 for the 8 diluted wastewater with HT sludge.

### 3.2.1. Hydrogen consumption tests

The lack of net volume of hydrogen gas evolved in fermentation tests could be due to an equivalent rate of hydrogen consumption via acetogenesis. When hydrogen was added to the headspace of wastewater with an HT sludge inoculum at a concentration of ~60%, the hydrogen was rapidly consumed when the initial pH was 6, 7, or 8 (Fig. 5), and acetate concentrations increased (data not shown). The abiotic control bottles without inoculum did not consume hydrogen in the headspace, and the biotic control bottles with nitrogen in the headspace did not produce hydrogen (data not shown). At a pH of 5, the rate of hydrogen consumption was substantially reduced, but as previously stated, tests at pH 5 showed little potential for hydrogen production. Thus, it appears that there is a great ability for the sample to consume hydrogen gas, making it difficult to recover small amounts of hydrogen gas under fermentation conditions.

### 3.2.2. Continuous flow reactor tests

Hydrogen production by fermentation was also examined in continuous culture to test the hypothesis that hydrogen production...
production might be achieved under conditions that would wash out hydrogen-consuming microorganisms. There was no sustained hydrogen production using either raw or autoclaved swine wastewater (diluted 2×) in a reactor operated under continuous culture conditions (data not shown). There was hydrogen initially produced when glucose was added during the reactor start up, but when the reactor was operated in continuous flow mode there was no hydrogen production under steady state conditions (data not shown).

4. Discussion

High COD wastewater such as swine wastewater can be used to produce hydrogen gas using an MEC, but not by fermentation. In fermentation tests, no hydrogen gas was recovered unless the wastewater was autoclaved, and even under those conditions the small amount of hydrogen gas that was evolved was consumed by the sample. In an MEC, hydrogen gas was evolved at a fast rate, and thus appreciable amounts of hydrogen gas could be recovered before it could be used by microorganisms in the wastewater. The rates of hydrogen gas production measured here were similar to those found in recent studies using acetate (Cheng and Logan, 2007; Call and Logan, 2008). Treatment efficiencies in MEC tests with swine wastewater ranged from 19 ± 15 to 72 ± 4% based on COD reduction. While this is a large range, it is similar to the range of treatment seen in more conventional digesters, which produce COD reductions from 28 to 87% (Chynoweth et al., 1998). This suggests that treating swine wastewater in an MEC may be a viable option for both producing hydrogen gas and reducing the COD.

Coulombic efficiencies are used to evaluate the percent of electrons from the COD that are transferred into current. The CE in the short-cycle tests were 29 ± 17% and 43 ± 2%, and 48 ± 9% and 70 ± 2% in the long-cycle tests. The lower CE in all experiments except for the long test with the undiluted waste (ND-L) indicated that a large percentage of electrons were not successfully transferred into current. A large percentage of the COD removed was therefore transferred to electron acceptors such as CO₂, stored in cells, or used for cell growth, as shown in Fig. 2. Sludge production may be problematic if electrons are shuttled into cell synthesis rather than current. Methane was produced in all tests, with 5 ± 2% to 21 ± 7% of the COD removed converted to methane. Less methane was recovered in tests with undiluted waste than with the diluted waste.

The use of the single-chamber design, in which the hydrogen is evolved into the wastewater, allows for a simple reactor design (compared to reactors with a membrane) and can achieve high hydrogen production rates. However, the lack of a membrane can reduce hydrogen recoveries due to hydrogen consumption by microorganisms in the wastewater. In the longest test using non-diluted wastewater, 70% of the electrons were recovered as current (CE) but only 29% of these electrons were successfully recovered as hydrogen gas (\(r_{H2, COD}\)). The increase in CE compared to the other tests may be due to reoxidation of hydrogen at the anode, which supplies electrons to the circuit without a concurrent COD reduction. In addition, acetogenesis may be occurring, as seen in the hydrogen consumption tests from the fermentation experiments. Acetate could either be oxidized or converted into methane. In contrast, tests conducted using only a single substrate (acetate) in a defined medium resulted in nearly 80% of the electrons recovered as hydrogen gas (same applied voltage of 0.5 V, and a comparable conductivity of 7.5 mS; Call and Logan, 2008). Hydrogen production rates here were 0.9 or 1.0 m³ m⁻³ day⁻¹ with the swine wastewater, compared to 1.5 m³ m⁻³ day⁻¹ for tests using acetate. The current densities using swine wastewater were only slightly lower (92 A m⁻³ for dilute wastewater, 109 A m⁻³ for non-diluted wastewater; Table 1) compared to 145 A m⁻³ for the Call and Logan (2008) study. Thus, it appears that using swine wastewater achieves good current densities but that hydrogen recovery is much more challenging with a wastewater containing a high number of hydrogen-consuming microorganisms. Despite these biological hydrogen losses, overall recoveries (\(r_{H2, COD}\)) were still between 17 ± 7% and 28 ± 6%, a range that is much higher than a previous MEC study with wastewater using a two-chamber system with a membrane separating the anode chamber from the cathode (Ditzig et al., 2007).

The observation that hydrogen was only minimally produced during fermentation using the autoclaved sample demonstrates that hydrogen production is intrinsically possible by this method, but that other factors limit hydrogen gas recovery. The lack of hydrogen recovery is concluded to be due to utilization of hydrogen by microorganisms in the wastewater and low hydrogen production rates. This conclusion is based on tests showing little hydrogen evolution from even autoclaved wastewater, and tests showing that hydrogen added to the gas phase is rapidly consumed by microorganisms in the wastewater. There are several factors that could have contributed to low hydrogen production. For example, hydrogen evolution could simply be due to a lack of suitable sugars in the wastewater, as most hydrogen evolved in high-rate hydrogen fermentation tests is a result of sugar in the sample. Only 4% of the nearly 7000 mg/L of total reducing sugar was recovered as hydrogen gas (data not shown) in the raw wastewater was soluble (evaluated using the phenol-sulfuric acid method for reducing sugars, Dubois et al., 1956). The addition of glucose to any wastewater sample stimulated hydrogen evolution (Fig. 4), but there was no hydrogen production without adding glucose or sterilizing the wastewater. Thus, slow kinetics of combined sugar hydrolysis could have limited hydrogen fermentation rates. It is also possible that ammonia inhibited hydrogen-producing bacteria from germinating or growing rapidly. However, the ammonia concentration in batch tests using full-strength wastewater was ~2000 mg-N/L, and using 2× diluted wastewater was ~1000 mg-N/L. These ammonia concentrations are below concentrations reported to inhibit either hydrogen production rates or yields (at pH of 5.2 or 6.2; Salerno et al., 2006), and below inhibitory levels reported for methanogenesis at the two pH values used in these tests (Gallert and Winter, 1997). Ammonia concentrations were the same in the autoclaved and untreated wastewater tests. As a result of all these factors, it appears that hydrogen recovery from swine wastewater will not be feasible by fermentation processes unless some breakthrough is made in changing the nature of the wastewater or the conditions for microbial growth that inhibit the utilization of the hydrogen by microorganisms in the wastewater.
4.1. Outlook for swine wastewater treatment using MECs

The use of MECs for wastewater treatment will depend on many factors including the cost of the materials, and the amount of energy needed. The cost of materials for large-scale treatment is not yet known. The energy efficiencies based on the energy value of the hydrogen gas produced compared to the electrical energy input were high here using the single-chamber MEC and wastewater, and thus from an energy standpoint MECs may be a promising method of treatment. Although MECs are unlikely to become cheaper than anaerobic digestion for high-strength wastewater applications due to capital costs for the electrodes, MECs can produce a high value product and may have a role in wastewater treatment in special circumstances where the hydrogen produced has a value that can offset the capital costs. Rozendal et al. (2008) offer a similar argument for electricity produced by MFCs. The efficiency of the short-cycle time reactor with non-diluted wastewater was 190 ± 39%. Energy efficiencies in other tests were similarly high when the total cycle time was short, primarily as a result of the low energy input from the power source over this shorter time. Note that the energy content of the organic matter is not included in this energy balance since it is “free” compared to the electrical energy needed, and thus the efficiency can be over 100%. The amount of energy provided by the power source per mass of COD removed was approximately 0.8 kWh/kg-COD. If all the electrons available in the removed COD (1 mol e−/8 g COD) had been successfully harnessed as current, 1.67 kWh/kg-COD would be required by the power source applying 0.5 V. However, since approximately 50% of the electrons from the COD did not produce current, the energy requirement in these experiments was about half that expected by an applied voltage of 0.5 V. These values for energy requirements for this process are within the 0.7–2 kWh/kg-COD range (Metcalf & Eddy Inc., 2003) needed for activated sludge treatment. However, in some of the MEC tests almost twice as much energy was created here in the form of hydrogen gas. Using the energy in the gas to power the system with a fuel cell, for example, could make the process nearly self-sustaining. Assuming 55% efficiency in a hydrogen fuel cell (for electricity production), using the hydrogen from the best-performing MECs in this study would provide ~10% more energy than the energy from combustion of both the hydrogen and methane (assuming 40% combustion efficiency). This might require clean up of the gas for use in a hydrogen fuel cell, a process which would need to be further examined. In the best scenario from this study, approximately 1 kWh/kg-COD removed could be achieved by combusting the hydrogen and methane. This is similar to the energy output achieved from combusting the methane produced by anaerobic digestion. The options for energy recovery should be further explored.

5. Conclusions

Swine wastewater was successfully treated while producing hydrogen gas using an MEC. In contrast, little hydrogen gas could be recovered from fermentation of the wastewater unless it was autoclaved, a procedure that would not be practical on a larger scale. In an MEC, the COD removals ranged from 19 ± 15 to 72 ± 4%, with hydrogen recoveries of 17 ± 7 to 28 ± 6% based on COD removed. The advantage of producing hydrogen gas in an MEC compared to other methods is the high-energy content and market value of the hydrogen. However, appreciable amounts of methane gas were also produced in this process, resulting in a biogas with several components. The use of this product gas for other purposes will need further evaluation to determine the overall practical nature of using an MEC for swine wastewater treatment.

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