Examination of microbial fuel cell start-up times with domestic wastewater and additional amendments

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Rapid startup of microbial fuel cells (MFCs) and other bioreactors is desirable when treating wastewaters. The startup time with unamended wastewater (118 h) was similar to that obtained by adding acetate or fumarate (110–115 h), and less than that with glucose (181 h) or Fe(III) (353 h). Initial current production took longer when phosphate buffer was added, with startup times increasing with concentration from 149 h (25 mM) to 251 h (50 mM) and 526 h (100 mM). Microbial communities that developed in the reactors contained Betaproteobacteria, Acetomicrobiomorpha norterae, and Chlorobium sp. Anode biomass densities ranged from 200 to 600 g/m² for all amendments except Fe(III) (1650 g/m²). Wastewater produced 91 mW/m², with the other MFCs producing 50 mW/m² (fumarate) to 103 mW/m² (Fe(III)) when amendments were removed. These experiments show that wastewater alone is sufficient to acclimate the reactor without the need for additional chemical amendments.

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1. Introduction

Microbial fuel cell (MFCs) are a promising approach for treating wastewater, as electricity is produced directly from the process of organics biodegradation (Logan, 2009; Logan, 2010). Many kinds of wastewaters have been tested in recent years, including synthetic, domestic and industrial wastewaters (He et al., 2005; Huang and Logan, 2008; Ahn and Logan, 2009). A number of factors should be addressed before MFC technologies can be applied at larger scales. Previous research has focused on anode and cathode materials, separators, and designs for scaling-up reactors. However, rapid start-up of any biological process used for wastewater treatment is desirable to avoid discharge of untreated wastewater.

Reported startup times for MFCs vary depending on the substrates examined and the reactor architecture, ranging from 10s of hours to several months (Feng et al., 2008; Liu et al., 2008). For example, more than 50 h was needed to obtain maximum voltages in two-chamber, air-cathode MFCs inoculated with anaerobic sludge (Kim et al., 2005). Liu and Logan (2004) demonstrated that the single-chamber, air-cathode MFC could produce a consistent maximum voltage after 140 h (0.32 V, 4 cycles) when inoculated with the effluent of a primary sedimentation tank, but a stacked MFC using a ferricyanide catholyte required 103 days following inoculation with a mixture of anaerobic and aerobic sludge (Aelterman et al., 2006). A cassette-electrode MFC with air-cathodes took 15 days before stable performance was achieved with a synthetic wastewater (Shimoyama et al., 2008). One of the most efficient methods for starting up a new MFC is to use the effluent from an existing reactor treating the same type of substrate (Jung and Regan, 2007; Chae et al., 2009; Kim et al., 2007). However, a large volume of pre-acclimated exoelectrogens may not be available for starting-up larger scale reactors. For example, a 1000 L pilot-scale microbial electrolysis cell (MEC) had to be inoculated with domestic wastewater to treat winery wastewater, and it required 60 days for start up (Cusick et al., 2011). Thus, more information is needed on how to start up larger reactors in the absence of a pre-acclimated inoculum. In the case of domestic wastewater treatment, it is not known if startup can be accelerated or subsequent performance can be improved through creating different conditions in the wastewater during the start up phase of operation.

Several approaches can be used to improve startup. First, additional substrates can be added since the wastewater strength is typically lower than that used in laboratory systems (~1 g/L chemical oxygen demand, COD). Second, the addition of specific alternate electron acceptors can be used to encourage the growth of known exoelectrogenic bacteria such as various Geobacter or Shewanella species. Fumarate or Fe(III) is often used to culture Geobacter sulfurreducens prior to inoculation into an MFC (Wang et al., 2010; Torres et al., 2009), although fumarate can also be used...
as a substrate by bacteria. Third, the conductivity of domestic wastewater is low (~1 mS/cm) compared to some buffered laboratory solutions (~7 mS/cm for 50 mM phosphate buffer). A low conductivity can limit current densities, and thus it may allow other non-exoelectrogenic bacteria to colonize the electrodes and inhibit growth of exoelectrogenic bacteria. Others have suggested electrochemical control of the system, such as setting the anode potential, might also work as a method to improve startup (Wang et al., 2009b).

In this study, the effects of different additives (electron donors, acceptors, and varied ionic strength buffers) to domestic wastewater were examined to try to improve startup times and power production. While the addition of substrates and buffers have been examined for power generation from specific chemicals (i.e. glucose and acetate), the use of additives to improve the long term operation of an MFC treating a domestic wastewater has not previously been examined. In addition to examining startup time and power, the microbial communities that developed in these reactors after relatively long-term operation was characterized.

2. Methods

2.1. Wastewater source

Domestic wastewater (primary clarifier effluent) was obtained from the Pennsylvania State University Wastewater Treatment Plant and used without any pretreatment as a control. Split wastewater samples were then amended with sodium acetate, sodium fumarate, Fe(III) or phosphate buffer (PB) at different concentrations shown in Table 1. Phosphate buffer and Fe(III) oxide (ferricydrite) was prepared as previously reported (Wang et al., 2010; Zuo et al., 2008).

2.2. MFC construction and operation

Single-chamber MFCs were constructed from media bottles (320 mL capacity, Corning Inc., NY) and a cathode electrode placed at the end of a side port (3.8 cm in diameter, projected cathode surface area of 4.9 cm²) and coated on the water facing side with a Pt catalyst (0.5 mg/cm²; 10% Pt) as previously described (Logan et al., 2007). Anode electrodes (1.5 cm × 9 cm, 27 cm²) were made of heat-treated carbon cloth (type A, BASF Fuel Cell, Inc., USA) (Wang et al., 2009a). All reactors were covered with aluminum foil to exclude light. MFC operation was divided into two stages: (1) startup with different amendments to the wastewater (~525 h); and (2) subsequent operation with only wastewater (~675 h). All experiments were carried out in duplicate at room temperature (23 ± 1 °C).

2.3. Electrochemical measurements and calculations

The voltages (V) across a 1000 Ω external resistance were recorded using a multimeter with a data acquisition system Model 2700 (Keithley Instruments, Cleveland, OH, USA). Power density curves were measured and normalized by the projected cathode surface area as previously described (Cheng and Logan, 2007).

2.4. Anode characterization

A 1.5 cm × 2 cm piece of the anode was cut off during start-up and analyzed for the bacterial community and biomass concentration. Anode biomass (1.5 cm × 1 cm) was measured in terms of protein concentration by the bicinechonic acid method (Sigma Chemical Co., USA) (Ishii et al., 2008; Zhang et al., 2009). Genomic DNA was extracted from the samples with a PowerSoil™ DNA Iso-
110 h using WW + acetate and the longest was 353 h using WW + Fe(III). The addition of glucose delayed startup to 181 h compared to 118 h with only WW. This result was consistent with previous reports that acetate and glucose had distinctly different times needed for current generation (Lee et al., 2008), with 5 days for acetate and 8 days with glucose.

All reactors produced electricity after the first cycle (Fig. 2A), with the cell maximum voltages obtained with a fixed resistance of 1000 Ω during start up decreasing in the order: WW + acetate > WW + fumarate > WW > WW + glucose > WW + Fe(III). After two cycles (270 h), all the maximum cell voltages exceeded 200 mV, with the highest one (261 mV) due to acetate addition (WW + 400 mg/L acetate). These results are consistent with those reported by others that acetate fed MFCs produce higher voltages than those fed glucose (Jung and Regan, 2007). It is well known that G. sulfurreducens is important for high power densities in MFCs, and that it grows through reduction of Fe(III) and fumarate (Ishii et al., 2008; Kiely et al., 2011). It was found here that wastewater amended with Fe(III) initially (first cycle) produced a much lower voltage (159 mV) than fumarate (303 mV). Lower voltages could be due to growth of G. sulfurreducens in solution using Fe(III) rather than on the anode, or to flocculation due to Fe(III) amendments as the COD decreased to less than 200 mg/L (see Table 1). Higher power densities with fumarate than Fe(III) may have been due to fumarate serving as an additional electron donor for anodic bacteria.

When reactors were switched to a feed of only WW, the maximum voltages remained in the range of 202–239 mV for cycle 3, and decreased to 176–227 mV in cycle 4. The lowest maximum voltages were produced in the reactors started up with WW and fumarate (176 mV).

### 3.2. Start-up with different PBS buffers

The addition of PBS (25, 50 or 100 mM) was detrimental to MFC startup times but in some cases increased power (Fig. 1). The startup times were 149 h for 25 mM PBS and 251 h for 50 mM PBS, and the reactor with 100 mM PBS failed to start up. The maximum voltages obtained with a fixed resistance of 1000 Ω, and with PBS added (cycle 2) decreased in the order: WW + 50 mM PBS (406 mV) > WW + 25 mM PBS (366 mV) > WW (231 mV) > WW + 100 mM PBS (8 mV) (Fig. 2B). When the PBS addition was discontinued and the reactors were fed only wastewater, the reactors originally amended with 100 mM PBS produced nearly as much power as the other MFCs (cycle 3 and cycle 4). The lower PBS concentrations likely increased power when they were added by increasing the conductivity of the wastewater. However, too large an increase in salinity (from ~1 mS/cm for WW to 12.8 mS/cm for 100 mM PBS) may have adversely affected bacteria and resulted in poor performance.

It is well known that 100 mM PBS buffer produces higher cell voltages than lower PBS concentrations (Logan et al., 2007; Min et al., 2008; Nam et al., 2010). However, relatively few studies have examined MFC startup times with WW. Here, the MFCs fed WW with 100 mM PBS failed to perform well, producing <20 mV even after 526 h. Feng et al. (2008) showed that the addition of 200 mM PBS (14.6 mS/cm) increased the performance of an MFC when it was added after startup of the reactor with brewery wastewater. This suggests that it might be necessary to first acclimate a reactor for power generation, and then to add PBS. However, it is clear that PBS addition during the startup phase is not beneficial for reducing startup times using domestic wastewater.

### 3.3. Power production after startup

When amendments were discontinued and the reactors were fed unamended wastewater from 526 h (cycle 3) to 1200 h (cycle 4), the maximum voltages produced were all similar (200–215 mV) (Fig. 2). This suggests that the anode biofilm had the same capacity for current generation at a high resistance regardless of the startup strategies.

Polarization data obtained after the startup period with unamended wastewater (at 1050 h during cycle 4) showed that different maximum power densities could be produced as a result of the different electron donor and acceptor amendments (Fig. 3A), but not with the PBS amendments (Fig. 3B). The MFC originally fed Fe(III) produced the highest maximum power density of $P_{\text{max}} = 103 \pm 2 \text{ mW/m}^2$, with the lowest power of $P_{\text{max}} = \text{...}$. 

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*Fig. 1. Amount of time for MFCs to reach a maximum voltage of 200 mV or more during startup (with indicated amendments).*

*Fig. 2. Start-up and operation of MFC using (A) different substrates, and (B) different PBS concentrations. (1) Startup stage: cycle 1 (0–270 h) and cycle 2 (270–526 h); (2) operation stage with unamended WW: cycle 3 (526–933 h) and cycle 4 (933–1200 h).*
50 ± 2 mW/m² produced for the MFC originally amended with fumarate. The MFC that was always fed only WW produced a maximum power of $P_{\text{max}} = 91 ± 22$ mW/m² that is not statistically different to that produced with the MFC originally amended with Fe(III). However, the reactors amended with Fe(III) showed much less variation in power production than those consistently fed WW (as shown by the size of the error bars in Fig. 3). The reactors originally amended with PBS all produced maximum power densities of 88–96 mW/m² once they were run on only WW, even the reactor that had a poor start up with 100 mM PBS.

These maximum power densities are within a reasonable range based on the specific WW and MFC architecture used here. Liu et al. (2008) achieved a higher $P_{\text{max}} = 148$ mW/m² with wastewater from this treatment plant by using a more optimized reactor configuration due to a higher cathode surface area per volume of reactor. With the same single-chamber bottle reactors $P_{\text{max}} = 300$ mW/m² was obtained by using 1 g/L of acetate and 50 mM PBS from anaerobic sludge (Logan et al., 2007). Using the same single-chamber bottle reactor inoculated from anaerobic sludge, $P_{\text{max}} = 197$ mW/m² was obtained with 1 g/L of acetate and 50 mM PBS solution after 2 weeks, but this declined to 135 mW/m² after 3 weeks (Ren et al., 2011).

It appears that among the electron donor and acceptor amendments examined here only Fe(III) showed any promise of affecting subsequent power generation. The MFC originally fed Fe(III) produced more consistent maximum power densities than the WW fed reactor, and the other amendments resulted in lower power production when switched to an unamended WW.

4.3. Polarization and power density curves after 1050 h operation with wastewater by using (A) different substrates and (B) different PBS concentrations during startup (0–526 h).

4.4. Biomass measurement

The biomass density on the anode after cycle 1 ranged from 60 to 100 µg/cm² based on protein measurements (Fig. 4). Biomass densities using WW + PBS (83 µg/cm²) or WW + Fe(III) (60 µg/cm²) are comparable to the report by Yi et al. (2009) using 10 mM acetate and G. sulfurreducens strain (27–57 µg/cm²) after ~100 h, but this was much lower than 1245 µg/cm² reported by Zhang et al. (2009) after 1005 h with 1 g/L acetate and 50 mM PBS. After being fed wastewater for 674 h, the biomass densities increased to 200–600 µg/cm², with the exception of 1650 µg/cm² obtained from the MFC originally fed WW + Fe(III). The addition of Fe may have resulted in deposition of iron on the anode, which could have enhanced the growth of iron-reducing bacteria (Kim et al., 2005). Although 1650 µg/cm² of the biofilm density was 2.5 times higher than that using different medium, the $P_{\text{max}} = 103 ± 2$ mW/m² with WW originally amended with Fe(III) during startup was only 10% higher than that obtained with WW during the whole time with WW ($P_{\text{max}} = 91 ± 22$ mW/m²).

3.5. Microbial community analyses in MFCs

Based on DGGE results (Supplementary material, Fig. S1) several of the most prominent bands were excised and sequenced (Table 2). Band 2 was identified as most similar to Acetanaerobium noterae and was common to all reactors, regardless of the influent substrate or the time point sampled. A band most similar to Acidovorax sp. was found to be present in all reactors except for the WW + 50 and 100 mM PBS. One band, with a 90% similarity to a Legionella sp. (Band 1), was found to be unique to the WW + Fe(III) amended reactor. Betaproteobacteria, A. noterae, and Chlorobium sp. were identified by DGGE analysis of several of the other darker bands after being fed only WW.

There was an apparent shift in the anode communities of all reactors, regardless of the initial amendment to the system, after being fed only WW. This is seen from the clustering of the samples during the amendment period in a different quadrant than the samples fed only wastewater in a PCA plot (Fig. 5). Thus, the sampling time was more important to the structure of the community than the specific amendment.

3.6. Implications for MFC startup

High current densities from different biodegradable substrates are dependent on the rapid and successful colonization of the anode by electrochemically active bacteria. The results here demonstrated that adding additional electron donors and acceptors did
Table 2
Phylogenetic identification of predominant 16S rRNA gene fragments from DGGE bands.

<table>
<thead>
<tr>
<th>Band</th>
<th>GenBank closest match (accession number)</th>
<th>Identity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Legionella sp. HB09011 (GU319886.1)</td>
<td>90</td>
</tr>
<tr>
<td>2</td>
<td>Acetanaerobium notae strain ATCC 35199</td>
<td>99</td>
</tr>
<tr>
<td></td>
<td>(GUS62448.1)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Acidovorax sp. smarlab 133815’ (AY903698.1)</td>
<td>98</td>
</tr>
<tr>
<td>4</td>
<td>Beta proteobacterium PAC94 (AY297809.1)</td>
<td>100</td>
</tr>
<tr>
<td>5</td>
<td>Acetanaerobium notae strain ATCC 35199</td>
<td>98</td>
</tr>
<tr>
<td></td>
<td>(GUS62448.1)</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Chlorobium sp. enrichment culture clone Sz-5</td>
<td>98</td>
</tr>
<tr>
<td></td>
<td>(FJ793792.1)</td>
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not modify the microbial communities as significantly as the run time, based on DGGE band patterns analyzed using PCA (Fig. 5).

Appendix A. Supplementary material

References


