Understanding Factors that Affect Microbial Fuel Cell Performance: Inoculum Characteristics and Methanogenesis

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Understanding Factors that Affect Microbial Fuel Cell Performance: Inoculum Characteristics and Methanogenesis

A Masters Project Presented

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Abstract

Microbial fuel cells (MFCs) are a promising approach to wastewater treatment that use anode-respiring bacteria (ARB) to oxidize organic matter and generate electric current. Although these devices have great potential, MFCs are not yet commercialized primarily due to their low power output at pilot scale. Past studies have hypothesized power production may be largely limited by high internal resistances and competing microbial metabolisms (Logan et al., 2008).

The source of inoculum used to build MFC communities has been demonstrated to significantly influence cell resistance and microbial dynamics (Sun et al., 2008; Chae et al., 2008). Studies that have shown these effects have generally focused on anode acclimation using air cathode MFCs. Presently, the effects of inoculum source on power production or startup times have not been explored in MFC designs that incorporate cathode-oxidizing biofilms. An objective of this research was to observe if inocula source and initial biomass concentration could influence startup times and power production of MFCs with biocathodes.

The role of initial biomass concentration was investigated by seeding identical reactors sets with inoculum from the same source at different VSS concentrations. The results of these tests showed that the initial VSS concentration did not strongly influence MFC startup times or power production. Identical reactors inoculated with raw primary effluent (0.24 g VSS/L) and diluted primary effluent (0.08 g VSS/L) both obtained steady-state power values of 120 µW ± 40 µW and stable cell potentials of 27 mV ± 5.0 mV after approximately 10 days of operation.

A direct comparison of three sources of mixed culture inoculums, at similar initial VSS concentrations, was performed by seeding the anode and cathode compartments of triplicate H-type MFCs and monitoring their performance in a recycled batch-fed mode for extended periods.
Inoculum sources included primary clarifier effluent from the Amherst WWTP, anaerobic digestate from Barstow Dairy Farms, and anode effluent from a pilot scale MFC. MFCs inoculated with anaerobic digestate or primary effluent achieved similar performance after 8-10 days of operation with steady-state power values of 150 µW ± 20 µW and stable cell potentials of 30 mV ± 5.0 mV. MFCs seeded with anode effluent obtained power values of 40 µW ± 5.0 µW and stable cell potentials of 10 mV ± 2.0 mV after 8-10 days of operation. The most efficient conversion of acetate to electricity was obtained by MFCs inoculated with anaerobic digestate that achieved efficiencies of 37 % ± 6% during periods of stable cell voltages. These efficiencies are low compared to other studies that commonly report values as high as 70% when using acetate as the sole electron donor in excess (Lee et al., 2008).

Many studies using mixed cultures have reported poor power efficiencies linked to competition between ARB and methanogens in the anode (Schaetzle et al., 2008). Past work has demonstrated nitrate dosing can effectively inhibit methanogenesis (Conrad et al., 1998). This inhibition approach is attractive for MFC wastewater treatment due to the potential availability of nitrate via nitrification. A separate objective of this research was to test the effectiveness of low dosing concentrations of nitrate (1 mg-N/L and 10 mg-N/L) on communities native to MFC anodes. Using anaerobic cultivation methods, bottle cultures were enriched for methanogens using inoculum from the anode of an operating MFC. After two batch cycles, test cultures were dosed with sodium nitrate at either 1 mg-N/L or 10 mg-N/L. In general, the 10 mg-N/L dosing suppressed methanogenesis longer than the 1 mg-N/L dosing. The 10 mg-N/L dosing scenario suppressed methane production for up to 7 days ± 2 days while the 1 mg-N/L dosing scenario inhibited samples for up to 3 days ± 1 day. Furthermore, cultures that contained graphite granules were generally inhibited for periods 1-2 days shorter than suspended growth cultures.
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1.0 Introduction

In recent years, society has expressed a growing concern for the dwindling availability of fossil fuels and the many detrimental environmental impacts associated with their use. A recent study conducted by the Energy Information Administration estimated that the U.S uses about 3.2 TW of energy per year, of which only 8% is derived from renewable sources (EIA, 2014). A large amount of energy is currently used for water infrastructure. About 1.0% of the USA’s total energy consumption (47 GW) can be attributed directly to wastewater treatment (EPA, 2014).

With these figures in mind, it is apparent that sustainable wastewater infrastructure is of great importance for reducing our reliance on non-renewable energy resources. Currently, efforts are being made to reduce energy consumption associated with wastewater treatment processes. One area that holds promise is the use of microbial fuel cells (MFCs) in secondary wastewater treatment. As part of a treatment system, influent organic matter (COD) is oxidized in the anode by anode-respiring bacteria (ARB) releasing protons, electrons, and carbon dioxide. The liberated electrons flow through a load bearing circuit to a cathode where they are either consumed in an abiotic chemical reaction or accepted by cathode-oxidizing bacteria that reduce a terminal electron acceptor such as oxygen or nitrate (Logan et.al, 2009; Butler et al., 2010).

Although studies have reported operation of pilot-scale MFCs, they have not yet been commercialized primarily due to low power output (Logan and Regan, 2008). Through recent work, great strides have been made in the optimization of MFC configuration to decrease internal resistances and improve power generation. In 1998, power densities were at best 0.1 mW/m² and within a decade reached levels high as 1.0 W/m² (Figure 1). More recent studies using air-cathodes have reported maximum power densities as high as 6.9 W/m² (Logan et al., 2015).
Figure 1- Power production for MFCs shown over time on the basis of published results. In less than a decade, power production by MFCs increased by several orders of magnitude. Power production continues to be limited by systems that have the cathode immersed in water [aqueous cathodes (red triangles) and sediment MFCs (green diamonds)]. Substantial power production has been possible by using air-cathode designs in which the cathode is exposed to air on one side and water on the other side (blue squares). In general, wastewaters have produced less power than systems using pure chemicals (glucose, acetate and cysteine in the examples shown; purple circles). Logan and Regan, 2009

As power densities continue to increase, it is expected that current densities will eventually become limited by the maximum rate of electron transfer that can be sustained by bacteria. Therefore, we should shift more focus to the optimization of microbial communities within MFCs. Achieving maximum power requires a better understanding of the bacteria that flourish and become dominant in these communities, the mechanisms by which bacteria transfer electrons to the electrode, and the ways in which bacteria interact in these systems.
2.0 Inoculation Experiments

2.1.1 Effects of Inoculum Source on Power Production

Currently, several species of ARB such as *Geobacter* and *Shewanella* have been identified and the electron transfer mechanisms they use are fairly well characterized (Figure 2) (Lovely et al., 2012). Inoculating MFCs with pure cultures of ARB, however, is impractical for MFC wastewater treatment due to their electron donor specificity and potential sensitivity to environmental changes such as pH and temperature. For these reasons, mixed cultures are often preferred over pure cultures to seed MFCs (Logan et al., 2006). Currently, the community dynamics of mixed cultures in MFCs are not well characterized after seeding or during long-term operation.

Figure 1 - Potential mechanisms for microorganisms to transfer electrons to electrodes. (a) Short-range electron transfer by microorganisms in close association with the electrode surface through redox-active proteins, such as c-type cytochromes associated with the outer cell surface or in the extracellular matrix. (b) Electron transfer via reduction of soluble electron shuttles released by the cell. Oxidized shuttle molecules are reduced at the outer cell surface, and the reduced shuttle molecules donate electrons to the electrode. (c) Long-range electron transport through a conductive biofilm via electrically conductive pili, accompanied by short-range electron transfer from the biofilm to the electron mediated by extracellular cytochromes as in panel a.

Figure 2 - Potential mechanisms for ARB electron transfer Lovely et al. 2012
One factor that encompasses these characteristics and has been demonstrated to influence performance is the source of the inoculum used to build communities. For example, Sun et al. (2008) found that MFCs with the same architecture achieved higher power densities (373 mW/m²) and lower internal resistances (38 Ω) when inoculated with a mixture of anaerobic sludge and wetland sediment than with anaerobic sludge (maximum power 214 mW/m², internal resistance 248 Ω) or wetland sediment (maximum power 324 mW/m², internal resistance 102 Ω), respectively (Sun et al., 2008). In a separate study, the performance of single chambered MFCs inoculated with activated sludge were compared to identical MFCs inoculated with anaerobic sludge from a lab scale reactor (Gao et al., 2014). During steady-state operation, activated sludge-inoculated MFCs arrived at 0.27 V with a maximum power density of 5.79 W/m³, while anaerobic sludge-inoculated MFCs reached 0.21 V with a maximum power density of 3.66 W/m³ (Gao et al., 2014). Microbial analyses of the anodic biofilms after acclimation showed that distinct microbial communities had developed from each of the inoculum sources including proteobacteria, acidobacteria, cyanobacteria and nitrospirae (Figure 3).

**Figure 3**- Composition and relative abundance of bacterial communities based 16S rDNA sequences. Pattern A indicates the composition and relative abundance of bacterial communities in phylum level. Pattern B is the composition and relative abundance of bacterial classes in phylum Proteobacteria. Os1, initial aerobic activated sludge inoculum; As1, initial anaerobic sludge inoculum; Os3 and As3, anodic biofilms of steady-running MFCs started up with aerobic activated sludge or anaerobic sludge, respectively. Gao et al., 2014.
These studies have revealed that inoculum source can influence steady-state MFC power production and demonstrate that a wide variety of microorganisms can thrive in MFCs. Presently, studies such as these have generally used air cathode MFCs to make comparisons of inocula source therefore, their results only considered acclimation of anodic biofilms with abiotic cathodes (Figure 4) (Lin et al., 2013, Song et al. 2011, Sun et al., 2008, Gao et al., 2014).

![Image of an MFC diagram](image.png)

**Figure 4**- Air cathode MFC- ARB transfers electrons obtained from an electron donor (glucose) to the anode electrode. During electron production protons are also produced in excess. These protons migrate through the cation exchange membrane (CEM) into the cathode chamber. The electrons flow from the anode through an external resistance (or load) to the cathode where they react with the final electron acceptor (oxygen) and protons. Logan et al., 2006

Biocathodes are of growing interest in MFC wastewater treatment due to their potential to incorporate denitrification into the treatment process (Figure 5) (Castro et al., 2014). Presently, few studies have observed the effects of different inoculum sources on power production or startup periods using MFCs seeded with mixed cultures in both the anode and cathode chambers.
2.1.2 Importance of Inoculum Characteristics on MFC Startup Times

Acclimation or startup can be defined as the establishment of a stable microbial community. When treating wastewater, rapid startup of microbial fuel cells (MFCs) is desirable to avoid discharge of untreated wastewater and reduce operational costs. Startup times of MFCs have been known to vary from tens of hours to several months (Feng et al., 2008; Liu et al., 2008). Using a single chamber batch-fed MFC, inoculated with primary effluent, Liu et al. (2007) found approximately six days was needed to obtain maximum voltages. With a similar configuration, a dual-chamber, batch fed MFC inoculated with anaerobic sludge, Kim et al. (2005) found only two days was needed to obtain maximum voltages. Although a direct comparison was not made, these findings suggest the characteristics of the inoculum may have affected startup times.

In early work, MFCs were commonly inoculated with pure cultures of anode-respiring bacteria (ARB), however, more recent efforts have shown MFCs can be inoculated with communities from a wide array of sources (Lovely et al., 2011). Torres et al. (2008) demonstrated that ARB could be enriched from diverse ecosystems including marshes, lake sediments, saline microbial mats, and anaerobic soils (Torres et al., 2008). Additionally, aerobic
or anaerobic sewage sludge alone has also been shown to be sufficient to acclimate MFCs (Sun et al., 2008). Due to its availability, many MFCs are commonly inoculated with wastewater from existing wastewater treatment plants (Logan et al., 2010; Torres et al., 2008; Sukkasem et al., 2007).

In contrast to pure cultures of known ARB, mixed cultures (such as those from the sources listed above) have shown to more effectively metabolize complex fuel compositions, be more robust to environmental changes and achieve substantially greater power densities (Schaetzle et al., 2008). However, the diversity of mixed communities often allows for non-ARB to occupy space on electrodes potentially limiting power generation efficiency and increasing startup times (Chae et al. 2009, 2010).

Many researchers have attempted to reduce startup times using methods that select for ARB in mixed cultures (Gao et al., 2014). Two-chambered MFCs were inoculated with anaerobic sewage sludge and subjected to various enrichment techniques including applying MFCs with biofilm scraped from existing reactors, enriching cultures by serial transfer in a ferric iron medium, and using iron oxide-coated electrodes (Kim et al., 2005). The results of these experiments showed that in most cases simply seeding cultures and allowing for spontaneous colonization was favored (Kim et al., 2005). In contrast to this study, startup times of anode communities have been shortened by applying a poised anode potential. An anode potential that was poised to +200 mV versus Ag/AgCl during startup resulted in MFCs requiring 24 less days to obtain similar current output as un-poised controls (Wang et al., 2007). Though possibly effective, using a poised potential requires a relatively large amount of energy. If scalable, this energy usage may limit MFC viability.
The most practical approach to acclimate large-scale MFCs is to use effluent from an existing pilot-scale or full-scale reactor that can potentially provide large quantities of mixed cultures containing exo-electrogens and subsequently, allow for spontaneous colonization (passive approach) (Jung and Regan, 2007, Chae et al., 2009 and Kim et al., 2007). To decrease startup times for this approach, the characteristics of the inocula used may be an important factor to consider. Using single-chambered, air-cathode MFCs, Lin et.al (2013) noted that MFCs inoculated with river sediment at 3.2 g VSS /L took about four days longer to startup, than identical MFCs inoculated with activated sludge initially at 22.4 g VSS/L (Lin et.al, 2013). From these results, it can be noted that the initial biomass concentration of the inocula may have affected startup times but differences in acclimation could have also been attributed to the inocula source. Currently, direct comparisons explicitly evaluating the effects of initial biomass concentration of inocula on MFC startup times and power production are lacking.

2.1.3 Objectives of Inoculation Experiments

Studies have suggested that inoculum source and quantity may impact startup times and power production of MFCs, however, a few potential areas of importance have been overlooked. Past efforts have not explicitly considered the effects of initial biomass concentration of inocula on startup times or power production. Additionally, studies that have compared inocula source have not considered power production and startup times in MFC designs that incorporate cathode-oxidizing biofilms.

A major objective of this research was to investigate if inocula characteristics such as source and initial biomass concentration can affect startup times and steady-state power production of dual-chambered MFCs with biocathodes. To evaluate the effects of inocula source, inoculum was obtained from several existing pilot and full-scale reactors capable of providing large volumes of mixed culture inoculum and their performance was examined in identical
MFCs for extended periods. These sources included the Amherst WWTP primary clarifier effluent, anaerobic digestate from Barstow Dairy Farms, and anode effluent from a pilot scale MFC. To examine the effects of initial biomass quantities of inoculum, two different initial VSS concentrations from the same source were evaluated on identical MFCs. For all inoculation experiments, initial VSS concentrations were adjusted to values similar to those of potential upstream sources in a full-scale treatment system. The methods used for these experiments as well as the results are described below.

2.2 Materials and Methods

2.2.1 Reactor Construction and Operation

Three identical MFCs were constructed in an H-configuration. MFCs consisted of an anode and cathode separated by a proton exchange membrane (Figure 6). MFCs were assembled from 250 mL Nalgene sample bottles. Each MFC consisted of two bottles connected by 1 in. ID PVC piping and a pipe junction (B&K 164-635HC) that contained a proton exchange membrane (Ultrex CMI-7000, SA = 1.78 cm$^2$) sandwiched in the middle

Figures 6 (Left) and 7 (Right)- H-type MFCs; anode and cathode separated by proton exchange membrane; 250 mL Nalgene Sampling Bottles filled with 200 grams of graphite granules
Influent ports and effluent ports were made in the base and top of each bottle, respectively. Ports were located on opposite ends of the bottle (Figure 7). All connections were sealed with a silicon-based sealant and dried overnight. Following this procedure, MFCs were filled with reverse osmosis (RO) water and leak tested. Once the integrity of each MFC was validated, 200 grams of graphite granules (EC 100 3/8x10, Graphite Sales, SA = 3.16 x 10^3 cm^2) were placed into each chamber. The remaining liquid volume in the electrode chambers was 160 mL. To create an anaerobic environment, each bottle was fitted with an airtight rubber stopper (Fisher Brand #6). Before operation, each stopper was cut with a small incision to allow wire to connect a graphite rod current collector in each chamber (Graphite Store, OD: 1 mm; L: 15 mm). The anode and cathode were connected with a 51 Ω resistor, across which cell voltage and power were measured. An Ag/AgCl reference electrode (+0.197 V vs. SHE) was placed in each cathode compartment on the twenty-fourth day of test 1 (MFC-A) and connected via a wire to a Keithley data acquisition multimeter (model 2700, Cleveland, Ohio).

Prior to each experimental run with a different inoculum, new graphite granules and current collectors were installed at the beginning of each test. Feed bottles were autoclaved or soaked in a 5 % by volume bleach solution overnight. Additionally, all pipe tubing, stoppers, and reactors were replaced or soaked in a 5% by volume bleach solution overnight and rinsed with soap thoroughly before use.

Inoculum was obtained from existing pilot and full-scale treatment process reactors that potentially contain large quantities of mixed cultures containing ARB. Anaerobic digestate was taken directly from the effluent of a mesophilic anaerobic digester treating dairy farm waste at Barstow Farms. Similarly, anode effluent was obtained directly from the anode effluent port of two-chambered, pilot-scale MFC treating complex organics. Wastewater was acquired by sampling the effluent of a primary clarifier at the Amherst WWTP.
Prior to seeding the conductivity, the pH, and temperature of the inoculum source was obtained using a pH meter equipped with a temperature/conductivity probe (Fisher Science Education). Following this procedure, the total suspended solids (TSS) and volatile suspended solids (VSS) of the inoculum were determined in accordance with Standard Methods (AWWA-1999). If needed, adjustments to the inoculum VSS concentration were made by diluting inoculum sample with RO water to test concentrations around 0.9 g VSS/L. After dilution, VSS was measured again to validate the test value. Following this procedure, the sample was sparged with 99.9% purity nitrogen gas at 45 psi for approximately thirty-five minutes. While sparging, inoculum was mixed with a stir-bar to ensure uniformity. Inoculum from the mixing bottle was dosed to both MFC chambers and feed bottles to form a 1:10 dilution with the total liquid present in the batch system.

**Table 1- Inoculum Source and Test Conditions**

<table>
<thead>
<tr>
<th>TEST GROUP</th>
<th>Source Name</th>
<th>Abbreviated Test Name</th>
<th>Source pH</th>
<th>Source Conductivity (µS)</th>
<th>Source Temperature (°C)</th>
<th>Initial gVSS/L in MFCs after dosing</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>MFC-Anode effluent</td>
<td>MFC-A</td>
<td>7.2</td>
<td>596</td>
<td>21.2</td>
<td>0.09</td>
</tr>
<tr>
<td>2</td>
<td>Primary Effluent-Amherst WWTP</td>
<td>WW</td>
<td>7.6</td>
<td>4190</td>
<td>24.6</td>
<td>0.24</td>
</tr>
<tr>
<td>3</td>
<td>Primary Effluent-Amherst WWTP</td>
<td>WW-Diluted</td>
<td>7.4</td>
<td>4569</td>
<td>22.0</td>
<td>0.08</td>
</tr>
<tr>
<td>4</td>
<td>Anaerobic Digestate-Barstow Farms</td>
<td>AD-Diluted</td>
<td>7.9</td>
<td>5040</td>
<td>37.0</td>
<td>0.09</td>
</tr>
</tbody>
</table>
**Table 2 - Common Microorganisms Found in Inoculum Sources**

<table>
<thead>
<tr>
<th>Inocula</th>
<th>Common Communities of Microbes Present</th>
<th>Common Metabolic Pathways</th>
</tr>
</thead>
<tbody>
<tr>
<td>MFC Anode Effluent</td>
<td>Anode-respiring Bacteria (ARB)</td>
<td>Iron- Reduction</td>
</tr>
<tr>
<td>Primary Effluent</td>
<td>Beta and Gammaproteobacteria - Acintobacteria, Bacteroidetes, Firmicutes</td>
<td>Wide array- Both Aerobic and Anaerobic processes</td>
</tr>
<tr>
<td>Anaerobic Sludge</td>
<td>Proteobacteria, Firmicutes, methanogens,</td>
<td>Methanogenesis, Fermentation</td>
</tr>
</tbody>
</table>

Lovely et al., 2011; Logan et al., 2005; Griffin et al, 1997 McLellan et al., 2010; Gao et al., 2014

Each MFC was operated in a recycled batch fed mode in which feed was recycled via a pump (Cole-Palmer Masterflex L/S) between a feed bottle and electrode chambers using 3/8” ID tubing (masterflex #16). To ensure liquid was not stagnant for extended periods and was uniformly distributed, the flow rate into each electrode chamber was set at 20 mL/min during the entire experiment. To limit phototrophic growth all reactors and feed bottles were covered in aluminum foil. All MFCs were stored in the laboratory at approximately 22 °C. In each MFC, anode and cathode chambers were fed from separate feed bottles in accordance with the recipes (Tables 3-5). The anode was fed a phosphate-buffered minimal growth media containing acetate and trace minerals (Table 3), while the cathode was supplied with a phosphate buffer minimal growth media containing nitrate and trace minerals (Table 4). All new feed was sparged with 99.9% purity N₂ at 25 psi for approximately thirty-five minutes. Each experiment was run until stable conditions were achieved indicated by repeatable peak power production over three feed changes (approximately three weeks).
Table 3- Anode Feed Recipe

<table>
<thead>
<tr>
<th>Compound</th>
<th>Na₂HPO₄ (dibasic)</th>
<th>KH₂PO₄ (monobasic)</th>
<th>NH₄Cl</th>
<th>MgCl₂</th>
<th>Final Potassium Acetate Concentration in Anode Compartment (500 mg COD /L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose (g/L)</td>
<td>1.386</td>
<td>0.849</td>
<td>0.050</td>
<td>0.040</td>
<td>0.831</td>
</tr>
</tbody>
</table>

Table 4- Cathode Feed Recipe

<table>
<thead>
<tr>
<th>Compound</th>
<th>Na₂HPO₄ (dibasic)</th>
<th>KH₂PO₄ (monobasic)</th>
<th>Na₂CO₃</th>
<th>MgCl₂</th>
<th>Final Sodium Nitrate Concentration in Cathode Compartment (40mg-N/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose (g/L)</td>
<td>0.710</td>
<td>1.50</td>
<td>.0378</td>
<td>0.050</td>
<td>0.244</td>
</tr>
</tbody>
</table>

Table 5- Calcium Iron Solution Recipe- 1ml/L growth media

<table>
<thead>
<tr>
<th>Compound</th>
<th>CaCl₂(2H₂O)</th>
<th>FeSO₄(7H₂O)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose (g/L)</td>
<td>1.00</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Table 6- Trace Mineral Solution Recipe- 1ml/L growth media

<table>
<thead>
<tr>
<th>Compound</th>
<th>ZnSO₄ (7H₂O)</th>
<th>MnCl₂ (4H₂O)</th>
<th>Na₂SeO₃</th>
<th>H₃BO₃</th>
<th>CoCl₂ (4H₂O)</th>
<th>Na₂MoO₄ (2H₂O)</th>
<th>CuCl₂ (2H₂O)</th>
<th>NiCl₂ (6H₂O)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose (g/L)</td>
<td>0.100</td>
<td>0.030</td>
<td>0.030</td>
<td>0.300</td>
<td>0.200</td>
<td>0.030</td>
<td>0.010</td>
<td>0.010</td>
</tr>
</tbody>
</table>

2.2.2 Analytical Methods

Acetate and nitrate concentrations were analyzed using an Metrohm 850 Professional Ion Chromatograph (Riverview, FL) equipped with an Metrosep A supp 5 anion column. Samples were collected directly from reactors as well as from feed bottles at the end of batch cycles. All
samples were filtered using 0.45 µm syringe filters and diluted with RO water to concentrations ranging between 0.2 and 10.0 mg/L to stay within machine detection limits.

For all experiments, cell voltage was measured every 15 minutes across an external resistor connecting the anode and cathode using a Kiethley digital multimeter data acquisition system (model 2700, Cleveland, Ohio). Furthermore, cathode electrode potentials were also measured every 15 minutes against an Ag/AgCl reference electrode (+0.197 vs. SHE) using the same device. Anode potentials presented in Figures 10 and 14 were calculated using equation EQ-2.1 (below).

\[
V_{\text{cell}} = V_{\text{Cathode}} - V_{\text{anode}}
\]

\( V_{\text{cell}} \) = Cell Potential (Anode vs. Cathode), mV
\( V_{\text{Cathode}} \) = Cathode Potential (Electrode vs. Ref. Electrode), mV
\( V_{\text{anode}} \) = Anode Potential, mV

Using the cell voltage data and acetate concentrations obtained from the IC methods listed above, Equation EQ-2.2 (below) was used to calculate coulombic efficiencies shown in Figures 11 and 16.

\[
CE = \frac{\Sigma_{i=1}^{T} V_{it}}{RFbMV_{\text{an}}}
\]

\( \Sigma_{i=1}^{T} V_{it} \) = Integral of Current for each time interval (i)
R = External Resistance (Ω)
F = Faraday's Constant (96,485 C/mol-e⁻)
b = Number of moles of electrons produced per mol of substrate (8 mol-e⁻/mol-acetate)
M = acetate concentration (M)
V_{an} = Anode Batch Volume (L)
CE = Coulombic Efficiency
At the end of each experiment, polarization curves were obtained using a Gamry Scientific Potentiostat. This module was operated in a two-electrode setup, swept at 1 mV/sec for a total of three cycles for each MFC. Polarization curves were obtained with MFCs in operation, at least once during steady conditions. Steady Conditions is defined here as the period for which average cell potentials of MFCs during the majority of a batch cycle remained constant ± 10%.

Power values presented in Figures 12 and 15 were calculated using equations EQ-2.3 and EQ-2.4 (below).

\[ V = IR \]  

\[ P = IV \]

\( V = \) Voltage Anode vs. Cathode (V)  
\( I = \) Current (A)  
\( R = \) Resistance (Ω)  

\( P = \) Power (W)  
\( I = \) Current (A)  
\( V = \) Voltage Anode vs. Cathode (V)
2.3 Results and Discussion

2.3.1 Effects of initial biomass concentration

The initial VSS concentration of inocula did not impact startup time or steady-state performance of MFCs. Although WW-diluted started with almost one third of the initial VSS concentration as WW, average cell potentials of both groups were similar during operation both achieving stable cell voltages of about 27 mV ± 5 mV after about 8-10 days of operation (Figure 8). Electrode potentials were also similar, both reaching cathode potentials of 62 mV ± 2 mV and anode potentials of 38 mV ± 9 mV after about 8-10 days of operation (Figure 9 and 10).

![Figure 8](image)

**Figure 8** - Comparison of Average Cell Potentials for WW (0.24 g VSS/L) and WW-Diluted (0.08 g VSS/L); MFCs over first six batch cycles; Values averaged from three identical acetate fed H-configured MFCs with denitrifying cathodes; Standard deviation (n=3) shown in error bars as gray shadow.

During periods of stable voltage, conversion of COD to electricity was also similar between WW and WW-diluted both achieving efficiencies of approximately 28% ± 4% (Figure 11). Moreover, power output between WW and WW-diluted was similar during steady conditions reaching values of 150μW ± 50μW and 100 μW ± 40 μW, respectively (Figure 12).
We hypothesize that ARB and COB may have been a small subset of the inoculum population so the increased biomass concentration had little affect on MFC performance.

**Figure 9**- Comparison of Average Cathode Potentials for WW (0.24 g VSS/L) and WW- Diluted (0.08 g VSS/L); MFCs over first five batch cycles; Values averaged from three identical acetate fed H-configured MFCs with denitrifying cathodes; Standard deviation (n=3) shown in gray shadow

**Figure 10**- Comparison of Average Anode Potentials for WW (0.24 g VSS/L) and WW- Diluted (0.08 g VSS/L); MFCs over first five batch cycles; Values averaged from three identical acetate fed H-configured MFCs with denitrifying cathodes; Standard deviation (n=3) shown in gray shadow
**Figure 11**- Coulumbic Efficiencies for WW (0.24 g VSS/L) and WW-diluted (0.08 g VSS/L); Values averaged from three identical acetate fed H- type MFCs, with denitrifying cathodes; Error shown as black bars.

**Figure 12**- Power during steady conditions for WW (0.24 g VSS/L) and Diluted WW (0.08 g VSS/L); Values averaged from three identical acetate fed H- type MFCs, with denitrifying cathodes during steady conditions; Standard deviation (n=3) shown as error bar.
2.3.2 Effects of Inocula Source on Startup Times and Performance

Inocula source did not greatly affect duration of startup times. MFCs inoculated with anode effluent from an operating MFC, anaerobic digestate, and primary effluent at similar initial VSS concentrations all reached stable cell potentials values after about 8-10 days of operation (Figure 13). Inocula source had some affect on power production of the MFCs. AD-diluted and WW-diluted achieved stable cell potentials about three times higher than MFC-A (Figure 13). Likewise, power values for AD-diluted and WW-diluted were approximately 2-3 times greater than MFC-A during steady-conditions (Figure 15).

![Figure 13- Cell Potentials – MFC-A (0.09 g VSS/L), WW-diluted (0.08 g VSS/L) and AD-diluted (0.09 g VSS/L); Potentials averaged from three identical acetate-fed MFCs with denitrifying cathodes shown during seven batch cycles; Standard deviation (n=3) shown in gray shadow and dashed lines](image)

Microbial communities collected from the anode effluent and used as inocula for MFC-A may not have been representative of communities in the anode biofilm. This consortium may have contained a variety of planktonic microorganisms as well as members of the anode biofilm that detached. These cells may not have been viable for anode colonization. Though the biomass of these cells was included in VSS concentrations, the actual starting number of viable cells may have been much less for MFC-A than for AD-diluted or WW-diluted.
The initial effect of each inoculum source on performance was sustained throughout operation. During steady conditions, WW-diluted and AD-diluted achieved similar cell potentials of about 30 mV ± 5 mV while MFC-A obtained potentials of about 10 mV ± 2 mV (Figure 13). The low cell voltages of MFC-A during steady operation were likely linked to characteristics of the biofilms that would have formed by this time.

The established communities in each MFC influenced the electrode potentials. AD-diluted achieved steady state anode potentials and cathode potentials of -1 mV ± 3 mV and 22 mV ± 5 mV while WW-diluted achieved steady state anode and cathode potentials of 31 mV ± 12 mV and 60 mV ± 9 mV (Figure 14). MFC-A obtained the most negative anode and cathode potentials of any group during steady conditions, approximately -10 mV ± 4 mV and 5 mV ± 1 mV, respectively (Figure 14).

![Figure 14](image.png)

**Figure 14**- Average Electrode Potentials of MFC-A (0.09 g VSS/L), WW-diluted (0.08 g VSS/L) and AD-diluted (0.09 g VSS/L) during steady conditions; Values averaged from three identical acetate fed MFCs with denitrifying cathodes; Standard deviation shown as bars

The more negative cathode potentials and lower cell voltages obtained by MFC-A during steady conditions may be partly attributed to the fact that these cultures were enriched in an anode prior to inoculation. The possible specialization of these cultures may have limited their
performance in cathode chambers. The potentially limited metabolisms of communities in MFC-A was likely responsible for the less efficient conversion of COD to electricity of this group compared to the other groups. During steady conditions, AD-diluted and WW-diluted achieved coulumbic efficiencies of about 36% ± 6% and 32% ± 3%, respectively (Figure 16). MFC-A obtained efficiencies of about 15% ± 2% (Figure 16). The source conditions for inoculums used in AD-diluted and WW-diluted typically allow for more diverse communities than MFC-A (Table 2). It is likely these cultures initially contained a greater variety of microbial metabolisms than MFC-A, which allowed them to better adapt to MFC environments (Table 2). It can be noted that AD-diluted and WW-diluted performed similarly throughout operation. This may be because the initial quantity of ARB and COB from each source was similar.

**Figure 15**- Power During Steady Conditions; Power of MFC-A (0.09 g VSS/L), WW-diluted (0.08 g VSS/L) and AD-diluted (0.09 g VSS/L); Values averaged from three identical acetate fed MFCs with denitrifying cathodes during steady conditions; Error bars shown in black.
Coulombic Efficiencies of MFC-A (0.09 g VSS/L), WW-diluted (0.08 g VSS/L) and AD-diluted (0.09 g VSS/L); Values averaged from three identical acetate fed MFCs with denitrifying cathodes shown over three batch cycles; Standard deviation (n=3) shown as error bars

2.4 Conclusions

The objectives of the inoculation experiments were to observe how inocula source and initial biomass concentration could affect startup times and MFC performance during steady conditions. The results of these tests showed that the initial VSS concentrations of inocula did not play a large role in startup times or performance. Identical reactors inoculated with raw primary effluent (0.24 g VSS/L) and diluted primary effluent (0.08 g VSS/L) both obtained stable cell potentials of 27 mV ± 5.0 mV approximately 8-10 days after inoculation. The source of inocula also seemed to have little affect on MFC startup times and performance. MFCs seeded with anaerobic digestate or primary effluent at similar VSS concentrations both achieved stable cell potentials of around 30 mV ± 5.0 mV after about 8-10 days of operation. MFCs inoculated with anode effluent obtained lower stable cell voltages of 10 mV ± 2.0 mV after 10 days. MFC-A was likely out performed by other sources due to a lack of initial viable cells and specialization caused by prior enrichment of cultures in an MFC anode.
3.0 Methanogenesis in MFC Communities

3.1 Implications of Methanogens in Anode Communities

MFC anode communities consist of a wide array of microorganisms. The diversity inherent in such communities yields advantages such as the ability to metabolize complex organics but can also allow for competing microbial metabolisms in MFCs (Schaetzle et al., 2008; Sun et al., 2009; Chae et al., 2010). In particular, a group of archaea known as methanogens have shown to significantly limit MFC power production (Call and Logan, 2008; Chae et al. 2008, 2009; Clauwaert and Verstraete, 2009; Freguia et al. 2007). In a MFC anode, methanogens can compete with ARB for electron donors as well as space on electrodes (Hamelers and Logan, 2012; Chae et al., 2010; Kim et al., 2005).

Some studies have explored methods to suppress methanogenesis within MFC anodes. Using two-chambered, air cathode MFCs inoculated with anaerobic sludge, Chae et al. (2010) examined the effectiveness of various environmental stresses such oxygen, low pH, low temperature, chemical dosing of 2-bromoethanesulfonate (BES), and variations in external resistances to inhibit methanogenesis in MFC anodes (Chae et al., 2010). The results of these tests showed that the most effective method was injection of 0.27 mM BES, which improved the conversion of COD to electricity by 35% (Chae et al., 2010). Although potentially effective, BES is an expensive chemical ($762/kg) making this approach unattractive for large-scale treatment (Chae et al., 2010).

Other approaches can inhibit methanogenesis but have not been tested in MFC anodes. The most well studied approaches involve the inhibition of methanogens by the promotion of competition with other microorganisms who metabolize a similar substrate such as acetate or hydrogen (Winfrey 1977, Abram 1978, Lovely 1982, Ward 1985). This concept was first presented in studies conducted by Winfrey et al. (1977) who demonstrated that the introduction
of sulfate to methanogenic communities allowed for sulfate-reducers to outcompete methanogens for electron donors in anoxic sediment samples from Lake Mendota (Winfrey et al., 1977). The superiority of the sulfate-reducers was linked to the observation that the organisms using electron acceptors with higher redox potentials had a higher affinity for electron donors. In a similar study conducted by Lovely and Goodwin (1998), this concept held true in the competition between bacteria using Mn⁴⁺, Fe³⁺, SO₄²⁻ and CH₄ in anoxic soil samples where concentrations of hydrogen were controlled (Lovely and Goodwin, 1988).

While approaches that promote competition have significantly inhibited methanogenesis, these methods could potentially affect power production in MFCs as ARB grow even slower than methanogens and would not be competitive for substrate with bacteria that use electron acceptors with higher redox potentials. Past studies have demonstrated that various chemical treatments, other than BES, are also effective at inhibiting methanogens (Chiu and Lee, 2001; Parameswaran et al., 2009). In a study conducted by Zhou et al. (2011), seven compounds including 2-bromoethanesulphonate (BES), propynoic acid (PA), nitroethane (NE), ethyl trans-2-butenoate (ETB), 2-nitroethanol (2NEOH), sodium nitrate (SN), and ethyl-2-butynoate (EB), were tested at final concentrations of 12 mM to inhibit methanogenesis in mixed communities. The most effective treatments included dosing of 2-nitroethanol and sodium nitrate, which decreased methane production by 70 to 99% compared to controls (Figure 17).
Figure 17- Amounts of biogas (A) and methane (B) produced per ml of *in vitro* culture after 48 h of incubation. PA, propynoic acid; BES, 2-bromoethanesulphonate; NE, nitroethane; ETB, ethyl *trans*-2-butenoate; 2NEOH, 2-nitroethanol; SN, sodium nitrate; EB, ethyl 2-butynote; C, control containing no inhibitor. Error bars indicate standard deviations, with different letters designating significant differences (Zhou et al., 2011)

Not much is known about the biochemical mechanisms involved in chemical treatments, however, studies have concluded some chemicals can be toxic to methanogens. In experiments conducted by Conrad et al. (1998), mixed cultures containing methanogens from anoxic rice field slurries were dosed with nitrate at approximately 140 mg-N/L. Monitoring the methane headspace concentrations of serum test vials after dosing, indicated that methanogenesis was inhibited for 24-27 days including periods in which all nitrate was reduced to nitrogen gas.

If using a MFC for wastewater treatment, a potential treatment for methanogens would be the introduction of nitrate produced via nitrification. High concentrations greater than 140 mg-N/L, as used in most inhibition studies, may allow denitrifiers to outcompete ARB as well as methanogens for electron donors leading to poor MFC performance (Conrad et.al, 1998; Zhou et al., 2011). Due to nitrate’s potential suppression of methanogens, dosing low-levels of nitrate in the anode of a MFC could potentially offer an effective method to suppress methanogens with
little impact on ARB. No studies have demonstrated if low-levels of nitrate can inhibit methanogenesis or studied its effectiveness on communities native to MFC environments.

3.1.2 Objectives of Methanogen Experiments

I hypothesize that dosing low-levels of nitrate (1 mg-N/L or 10 mg-N/L) can effectively inhibit methanogens enriched from MFC anodes. To test this hypothesis, anaerobic techniques were used to assemble batch cultures that selected for methanogens from communities obtained from the anode effluent of a pilot-scale MFC. Over extended periods, methane production of each culture was monitored before and after nitrate dosing. A summary of the experimental design, methods and results are provided in the subsequent section.

3.2 Experimental Design and Methanogen Cultivation Methods

3.2.1 Experimental Design

A total of 22 cultivation bottles were assembled to select for methanogens in mixed cultures obtained from the anode effluent of an operating MFC (Figure 18). One set of 12 bottles was incubated at room temperature (22°C) while another set of 10 was stored at 37°C. The room temperature set consisted of 5 identical bottles with graphite granules, 5 identical bottles without graphite granules (suspended growth) and 2 bottles with plastic media. The high temperature set consisted of 5 identical bottles with graphite granules and 5 identical bottles without graphite granules (suspended growth). All bottles were inoculated with anode effluent from an existing organic fed- MFC and filled with a phosphate-buffered minimal growth media containing acetate prior to incubation. The methane production as well as the electron donor consumption were monitored over the course of three batch cycles. In the third batch cycle, sodium nitrate was introduced to duplicate test bottles at concentrations of 1 mg-N/L and 10 mg-N/L. Within each set, duplicate bottles with graphite and duplicate bottles in suspended conditions were dosed at
each of the concentrations. Control groups consisted of one bottle with graphite and one bottle in suspended conditions that were not dosed in each set.

![Diagram of Experimental Design](image)

**Figure 18**-Summary of Experimental Design-Design Tree showing testing conditions of cultivation bottles; number of bottles under given conditions shown in parenthesis

3.2.2 Cultivation Methods

The assembly of the cultivations used in this study was done in accordance with the following procedure. First, 150 mL serum bottles were plugged with septa (Bellco Glass, 20mm 2048-11800), capped, and vacuumed. Graphite granules or plastic media were rinsed with RO water and added to a fraction of the bottles prior to being plugged (**Figure 18**). Next, nitrogen gas
(N₂) was used to purge air out of the bottles. The bottles were filled with 100 mL of a phosphate buffered minimal growth media containing 1 g/L acetate and, 1 mL/L calcium iron and trace mineral solutions (Tables 3-6). After the media was added, the bottles were autoclaved and 1mL of cysteine was added to each bottle to remove any residual oxygen. Inoculum from an operating MFC anode effluent was sparged with N₂ gas and added to test bottles in a 1:10 dilution with the growth media. High temperature bottles were stored in an incubator-shaker at 37 °C and room temperature tests were stored in the laboratory on a shaker at approximately 22 °C. All bottles were sealed in aluminum foil to limit phototrophic growth.

Batch cycles lasted on average six weeks. In general, liquid samples of approximately 0.75 mL were obtained from each culture bi-weekly to access the residual acetate levels. Feed changes were conducted when acetate concentrations in the majority of test bottles dropped below 50 mg/L. Changes were conducted by removing half the liquid volume of each bottle and replacing it with fresh concentrated growth media containing 2 g /L acetate to return the concentration to approximately 1 g /L acetate. The headspace of each bottle was vacuumed and replaced with pure N₂.

In the third batch cycle, sodium nitrate was introduced to culture bottles to produce final concentrations of approximately 1 mg-N/L or 10 mg-N/L. During the final feed change, three solutions were prepared; one a fresh concentrated growth media and two others amended with sodium nitrate. After removing half the liquid volume of each bottle, new media was injected, to produce final concentrations of approximately 1 g COD/L and either 1 mg-N/L and 10 mg-N/L. Following this procedure the existing headspace was vacuumed and replace with N₂. Cultures were vigorously mixed by hand and returned to incubation.
Table 7 - Motivations for each batch cycle

<table>
<thead>
<tr>
<th>Batch Cycle</th>
<th>Purpose(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Enrich for Methanogens; Compare Cultures with Graphite and Without</td>
</tr>
<tr>
<td>2</td>
<td>Further compare methane production of cultures; Gather methane production data prior to nitrate introduction</td>
</tr>
<tr>
<td>3</td>
<td>Introduce nitrate at beginning of cycle; Observe methane production</td>
</tr>
</tbody>
</table>

3.2.3 Analytical Methods

Methane headspace concentrations were monitored throughout the study using an Agilent 7890A Gas chromatograph (GC) equipped with an HP-PLOT-Q column and Thermal Conductivity Detector (TCD) heated to 200 °C. In general, 250 µl gas samples were extracted from the headspace of cultivation bottles at least every other day during a batch cycle. Immediately after sampling, gas samples were injected to the GC and analyzed for 15 minutes at an isothermal oven temperature of 60 °C. The peak areas of the samples were fitted to a six point standard curve obtained by analyzing pure methane samples diluted with nitrogen gas to final methane concentrations of 100 %, 50 %, 25 %, 10 %, 1 %, 0.5 %.

During the course of each batch, acetate and nitrate concentrations were monitored using an Metrohm 850 Professional Ion Chromatograph (Riverview, FL) equipped with an Metrosep A supp 5 anion column. In general, samples were taken directly from each test bottle on a bi-weekly basis. Prior to testing, all samples were filtered using 0.45 µm syringe filters and diluted with RO water to concentrations ranging between 0.2 and 10.0 mg/L.
3.3 Results and Discussion

3.3.1 Sodium Nitrate Inhibition

The introduction of low-levels of nitrate generally suppressed methane production and acetate consumption of bottle cultures (Figures 19 and 21). These effects were observed at both the 1 mg-N/L and 10 mg-N/L dosing concentrations, indicating inhibition under these conditions.

Figure 19- Duration of Inhibition- Average of duplicate cultures dosed with different concentrations of sodium nitrate at the beginning of final batch; sample headspaces tested every twenty-four hours; Inhibition defined as the cease of methane production; period in which no methane headspace concentrations were detected 5% greater than initial values at start of batch cycle

As expected, control groups without nitrate performed similarly to the batch cycles prior to nitrate dosing indicated by the amounts of methane produced (Figure 19). In the 1mg-N/L dosing scenario, four of the eight test cultures did not produce methane for periods ranging from one to three days (Figure 19). In general, graphite samples were less affected than suspended samples as both suspended samples at room temperature were inhibited for 2-3 days while only

![Graph showing duration of inhibition](image-url)
one of the graphite samples was inhibited under the same conditions (Figure 19). Similarly at the high incubation temperature (37°C), one of the suspended samples was inhibited for 1 day while none of the graphite samples were inhibited.

In the 10 mg-N/L dosing scheme, seven of the eight test cultures showed inhibition for periods ranging from one to seven days (Figure 19). Once again, graphite samples were more resilient than suspended samples as the only sample that was not inhibited was a graphite sample incubated at 37°C. Moreover, graphite samples were typically affected for shorter periods than suspended samples (Figure 19). It is likely since graphite communities were able to form biofilms, members closest to the substratum may have been protected from the effects of nitrate and continued methanogenesis, leading to the shorter observed inhibition periods (Figures 19). Large amounts of nitrate were consumed during inhibition periods, suggesting competition for electron donors between methanogens and denitrifiers as a possible inhibition mechanism (Figure 20).

![Figure 20- Nitrate Degradation; Amount of nitrate consumed during the first five days after dosing; Averaged from duplicate cultivation bottles dosed at 10 mg-N/L; Standard Deviation (n=2) shown in Error bars](image)
3.3.2 Enhanced Methane Generation

Prior to nitrate dosing, cultivations with graphite granules consistently achieved greater methane headspace concentrations than those in suspended conditions (Figure 22 and 23). At both incubation temperatures cultures that contained graphite achieved peak methane headspace concentrations almost 50% greater than those in suspended conditions during the second batch cycle (Figure 22). Furthermore, the amounts of acetate consumed by graphite samples at both incubation temperatures were more than 100 mg/L greater than suspended samples during the first five days of methane production (Figure 24).
Cultivations containing plastic media with similar surface areas as graphite samples were monitored for one batch cycle. These cultures performed similarly to suspended growth cultures reaching peak methane headspace concentrations of around 7% ± 2% (Figure 23). Samples that contained graphite generated more methane, achieving methane headspace concentrations as high as 25%. ± 8%. These observations suggested that the presence of graphite might have enhanced methane production (Figure 23). The presence of conductive materials, such as graphite, has been shown to enhance symbiotic relationships in methanogenic cultures via direct interspecies electron transfer (DIET) (Zhao et al., 2015).

Studies have demonstrated that interspecies electron transfer plays a crucial role in the metabolisms of methanogenic communities (Rotaru et al., 2014). A notable pathway for interspecies electron transfer is H₂ interspecies transfer (HIT), in which electron-donating microorganism reduce protons to H₂ and methanogens oxidize the H₂ with the reduction of carbon dioxide to methane (Rotaru et al., 2014). Recent studies conducted by Lovely et al. (2014)
demonstrated that direct interspecies electron transfer (DIET) is an effective alternative to HIT. In this process, microorganisms such as *Methanosaeta (Methanotrix) harundinacea*, can directly accept electrons from *Geobacter metallireducens*, a known ARB, for the reduction of carbon dioxide to methane (Lovely et al., 2014). Graphite is a preferred electrode material and has been used in geo-batteries to transmit electrons between anaerobic to oxic zones for distances up to 1km in the subsurface (Bigalke et al., 1997). It is possible that the presence of graphite within the batch cultures presented here allowed for DIET to take place and generate greater methane production and acetate consumption. In short, it is hypothesized that ARB oxidized acetate to produce carbon dioxide and electrons. The freed electrons were transferred to methanogens via DIET where methanogens accepted electrons for the reduction of carbon dioxide to methane. This in conjunction with conventional acetoclastic methanogenesis may have allowed for the greater methane production of cultures exposed to graphite.

**Figure 23**- Batch Cycle 1- Average methane headspace concentrations of five identical cultivation bottles with graphite granules, duplicate cultivations with Plastic Media, and five identical cultivation bottles suspended at room temperature; Error shown in bars
3.4 Conclusions

The objectives of the methanogen experiments were to test the effectiveness of 1 mg-N/L and 10 mg-N/L of nitrate to suppress methanogenesis in communities enriched from MFC anodes. These experiments demonstrated that both 1 mg-N/L and 10 mg-N/L of nitrate could effectively inhibit methanogenesis. The 10 mg-N/L dosing scenario, suppressed methanogenesis for up to a week while the low dosing of 1 mg-N/L suppressed methane production for at most three days. In general, graphite cultures were inhibited for shorter periods than suspended cultures.

4.0 Future Work

The goals of the inoculation experiments were to observe how inocula source and initial biomass concentration could affect startup times and MFC performance during steady conditions. The results of these tests showed that the initial VSS concentrations of inocula did not play a significant role in startup times or performance. Further work is needed to explore a greater range of initial biomass concentrations to better understand this effect.
The source of inocula also seemed to have little affect on MFC startup times and performance. It seems seeding MFCs using any diverse inocula source that can provide ARB may be acceptable, as it doesn’t seem to lead to large differences in startup times or performance. More work needs to be done in this area to identify the optimal groups to select for, the mechanisms by which they work together and common sources that potentially have large volumes of them available.

The objectives of the methanogen experiments were to test the effectiveness of 1 mg-N/L and 10 mg-N/L of nitrate to suppress methanogenesis in communities native to MFC anodes. These experiments demonstrated low doses (1 mg-N/L or 10 mg-N/L) of nitrate could effectively inhibit methanogenesis. If using nitrate for inhibition in a continuous flow MFC, it would potentially be appropriate to dose anode communities at 1 mg-N/L for short mean-hydraulic retention times (HRT) of 1-2 days. It should be noted that significant inhibition resilience was observed in cultures attached to graphite samples compared to suspended conditions, thus HRT is not the only parameter that should be taken into account to determine dosing. More work should be done to investigate the effectiveness of low levels of nitrate in MFCs operating in continuous flow. Furthermore, future studies should focus on better characterizing the mechanisms by which nitrate can suppress methanogenesis in mixed cultures. Although this research addressed important initiatives, many questions still need to be answered for the optimization of microbial communities within MFCs. MFCs are a very promising approach to sustainable wastewater treatment and have the potential to greatly reduce our future energy dependencies.
5.0 References


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