MICROBIAL FUEL CELL OPERATION AND USE WITH ANAEROBIC DIGESTION FOR POWER PRODUCTION FROM DAIRY MANURE

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ABSTRACT

As the world's population approaches seven billion people, it is vital to find methods of livestock production, energy generation, and organic waste utilization that are sustainable for the future. Livestock manure has long been valued as a fertilizer and soil amendment, and more recently as a methane producer for heat and power via anaerobic digestion (AD). Microbial fuel cells (MFC) may offer an additional opportunity to gain value through direct electrical generation with little reduction in the manure's soil building value, while concurrently reducing the manure's pollution potential.

We demonstrate that simple lab-scale MFCs capable of electrical production can be used with dairy manure and the microorganisms needed for electrical generation are endogenously present. The MFCs had an average internal resistance of 136 ohms. The MFC system was operated with various raw manure concentrations (0-100 g/L COD at 20 ^{o}C), generating a maximum power density of 138 ± 19 mW/m^2 (COD 100 g/L). Power production was proportional to manure strength over this COD range. The MFCs where tested at 20, 37, and 55 ^{o}C. Average total Coulombs (C) captured increased from 18.8±1.3 to 40.5±2.4 C, from 20 to 37 ^{o}C but fell to less than 1.42 C at 55 ^{o}C, indicating that the electrochemically active bacteria are possibly inhibited and killed at thermophillic temperatures. Coulombic efficiency also increased from 3.4±1.2% to 5.8±1.4% when the temperature was increased from 20 to 37 ^{o}C.

MFCs were combined with AD. AD bottles were measured for total biogas production and composition during a 45 day period at 37 ^{o}C. Operating a MFC before AD did not have a statistically significant impact on the amount of biogas produced.

However, the MFC operation did affect the timing and rate of biogas production for some of the samples. Biogas production reached a threshold of 5mL as many as 8 days sooner in the bottles that where first operated in a MFC. The maximum slope of the biogas curves was decreased by 55% from an average of 2.91 to 1.31 mL/day with prior operation in an MFC, indicating that the peak production rate was lower. The effect of AD digestion on the MFC performance was more significant with a 65% decrease in the total Coulombs captured when AD was completed prior to use in a MFC. To compare the two processes, the respective performance of the two processes was determined by Coulombic efficiency and energy captured. The MFCs reached a maximum of 7% Coulombic efficiency while the AD tests were all over 100% (indicating COD destruction was underestimated). Total energy capture for the MFCs was less than 1 Joule while with the AD tests it was 120-220 J. Both these measures indicate that AD is currently the more efficient process at converting biodegradable substrate into energy. However, with further research and development, MFCs show promise if applied in situations that fit their unique benefits, such as operation at temperatures and COD concentrations below that needed for AD. Examples include ambient temperature manure storage ponds and post-digestion substrates.

BIOGRAPHICAL SKETCH

Crystal Powers grew up on a small farm in South Central Nebraska which inspired her love of agriculture. She received her undergraduate degree in Biological Systems Engineering with minors in Communication Studies and Political Science from the University of Nebraska – Lincoln in 2005. During her undergraduate studies, Crystal studied for a semester in Central Europe, and had internships for the USDA Agricultural Research Service, the City of Delaware Ohio, Pfizer animal pharmaceuticals, and was a research assistant for the Biological Systems Engineering Department at UNL. She is currently a second year Masters student at Cornell University in Biological and Environmental Engineering, and has completed a minor in Systems Engineering.

To my husband William and my Family

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CHAPTER 1

INTRODUCTION

As the world's population approaches seven billion people, it is vital to find methods of livestock production, energy generation, and organic waste utilization that are sustainable for the future. "The building of a sustainable society will require reduction of dependency on fossil fuels and lowering of the amount of pollution that is generated. Waste treatment is an area in which these two goals can be addressed simultaneously. As a result, there has been a paradigm shift recently, from disposing of waste to using it" (Angenent et al. 2004).

Agricultural manures from animal confinements are ideal candidates as bioenergy feedstocks because they contain high levels of easily degradable organic material. Modern livestock agriculture has led to an increase in concentrated sources of manure. Currently there are over 75,000 total dairy operations with over 9 million head of cattle (NASS 2006). Each cow produces on average, 112 pounds of manure per day (EPA, 1999), or over 180 million tons of dairy manure annually. This manure has long been valued as a fertilizer and soil amendment, but can lead to significant air and water pollution challenges as well. Excess nutrients (nitrogen and phosphorous), organic matter, sediments, hormones, antibiotics, and pathogens can leach or runoff impairing water bodies. The release of odor, carbon dioxide, methane, ammonia, and nitrous oxide can also degrade air quality. Livestock manure storage is estimated to be responsible for two percent of greenhouse gas emissions worldwide (FAO 2006). Most of this is in the form of methane which has a global warming potential 21 times more than carbon dioxide. In the United States, the EPA estimates methane emissions from livestock manure management accounts for 10% of total 1997 U.S. methane

emissions (Figure 1-1) of which 27% come from dairy operations (EPA 1999). This number continues to increase due to the increasing size of farms and use of liquid storage for manure management. "Livestock's contribution to environmental problems is on a massive scale and its potential contribution to their solution is equally large. The impact is so significant that it needs to be addressed with urgency" (FAO 2006).

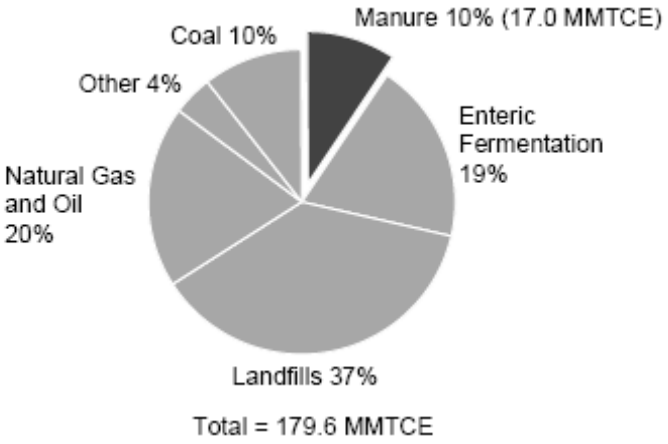


Figure 1-1. Percent of methane emissions in 1997 in Million Metric tons of Carbon Equivalents - MMTCE (EPA 1999).

Anaerobic digestion (AD) is a leading technology option in manure treatment, offering odor control, pollution reduction, and renewable energy production while maintaining the fertilizer value of the manure. AD occurs when organic substrates are degraded by an anaerobic microbial community that produces biogas, primarily a mixture of methane and carbon dioxide. This biogas can then be captured and used for energy production. In the United States, nearly 200 AD systems were currently operating or planned in 2006 (EPA, 2007). By capturing the biogas produced from manure storage, these systems have methane emission reductions in 2006 alone of approximately 80,000 metric tons and energy generation of about 275 million kWh (EPA, 2007).

Microbial fuel cells (MFCs) may be another option to gain value from the livestock manures through electricity generation while concurrently reducing pollution potential and maintaining fertilizer value. MFCs operate by capturing the direct electricity generation when electrochemically active bacteria breakdown organic substrates. MFCs have not previously been operated with dairy manure, however, they have been shown to reduce chemical oxygen demand (COD) of domestic wastewater by 94% (Cheng et al. 2006) and in swine manure slurries by 90%, while decreasing ammonia 83% and not significantly changing nitrogen levels (Min et al. 2005b) with concurrent electricity production. In MFCs, energy efficiencies range from 2% to 50% or more when easily biodegradable substrates such as organic acids or glucose are used. As a basis for comparison, the electric energy efficiency for thermal conversion of methane is approximately 10-30% when used in a conventional diesel engine-generator set, but can range as high as 40-50% when converted in a fuel cell (Rabaey et al. 2003, Liu and Logan 2004).

MFCs have been shown to operate under conditions similar to those found in dairy manure storage. The highest electric current observed thus far is generated at pH 7 (Gil et al. 2003), and dairy manure is an excellent buffer, maintaining near neutral pH levels. Also, MFCs operate well at lower temperatures than anaerobic digestion. For example, power output was reduced only 9% when temperature was decreased from 32 to 20°C (Liu et al. 2005a), which would be advantageous with manure storage systems that are maintained at ambient temperatures. This is similar to results found with AD systems where methane yield at lower temperatures (20-25°C) and increased detention time approaches that of conventional digestion at higher temperatures (35°C) and shorter detention times (Stevens and Schulte 1979).

MFCs have also been shown to utilize a variety of individual substrates found in dairy manure: glucose, acetate (Liu et al. 2005b), cysteine (Logan et al. 2005),

proteins (Heilmann and Logan 2005), lignocellulose (Rismani-Yazdi et al. 2006), as well as complex substrates such as domestic wastewater (Cheng et al 2006), swine manure slurry (Min et al. 2005b), landfill leachate (Frew and Christy 2006), and seafloor sediments (Tender et al. 2002).

Bacteria which are useful in microbial fuel cell operation have the ability to transfer electrons to an electrode (anode), as a terminal electron acceptor (Rabaey et al. 2004) and are classified as electrochemically active. Some of these species have been used in pure culture to generate electricity in MFCs. However, they have relatively low energy transfer efficiency compared to mixed microbial communities endogenous to wastewater, marine sediments, and livestock manures (Rabaey et al. 2003). MFCs that make use of mixed bacterial cultures have some important advantages over MFCs driven by pure cultures: higher resistance to process disturbances, higher substrate consumption rates, lower substrate specificity, and higher power output (Rabaey, K., G. Lissens, and W. Verstraete 2005).

The combination of anaerobic digestion and microbial fuel cells may offer further potential than either of these systems alone by taking advantage of the benefits of each technology. Anaerobic digestion and MFCs both use a mixed microbial community that is selected according to function. In AD, the microorganisms needed for eventual breakdown to methane is determined by a combination of microbial community dynamics and human control of input conditions and temperature, while in MFCs the community is selected for their ability to transfer electrons to the anode. This is well-suited to the non-sterile, ever-changing, complex environment of wastewater treatment. Also, the products from these bioprocesses can be easily separated as gases or bioelectricity (Angenent et al. 2004).

From a microbial perspective, these processes are competitive, using many of the same substrates. However, upon further inspection, these processes can be

complementary technologies. Anaerobic digestion can be applied to treat high strength substrates, with industrial scale feasibility, high throughput, relatively low cost, and high bioconversion efficiency. In the case of MFCs, application niches can be found in the area of treating low concentration COD substrates and at low temperatures (10–20 °C), where AD does not function well (Pham et al. 2006). MFCs also offer their own unique advantages: direct conversion of substrate energy to electricity enabling high conversion efficiency. For example, from every kilogram of biodegradable waste, approximately 1 kWh of electricity and 2 kWh of heat are produced during anaerobic digestion from biogas, with a power density of about 400 W/m^3 when used to treat 5-25 kg of COD per cubic meter per day, while a microbial fuel cell can theoretically deliver 3 kWh for every kilogram of organic matter in one single fermentative step. However, the current generated by MFCs, up to now, has not exceeded 0.1 Amp, with an average power density of about 40 W/m^3. Recently, stacked configurations of MFCs have reached power densities of 250 W/m^3, implying that an improvement of MFC performance is possible (Pham et al. 2006). Other advantages of a MFC include not requiring off-gas treatment as they primarily release carbon dioxide and normally have no waste heat, and they can operate on substrates not available to AD, such as sulfides (Rabaey et al. 2006).

For these reasons, this research sought to explore the use of microbial fuel cells with dairy manure and to determine if a MFC can operate efficiently in conjunction with anaerobic digestion. If these processes can be used concurrently, more energy recovery and efficient wastewater treatment may occur than from either process used alone.

CHAPTER 2

LITERATURE REVIEW

Microbial Fuel Cell Overview

A Microbial Fuel Cell (MFC) is a device that uses bacteria to generate electricity from the breakdown of organic substrates. Bacteria gain energy for metabolism by transferring electrons from an electron donor, such as glucose or acetate, to an electron acceptor, such as oxygen. The anode electrode of a MFC takes the place of the bacteria's typical electron acceptor, moving the electrons into a circuit, through a resistor, to the cathode electrode of the MFC, generating electricity. Protons diffuse from the anode and join with oxygen to form water at the cathode completing the reaction. This process is shown in Figure 2-1.

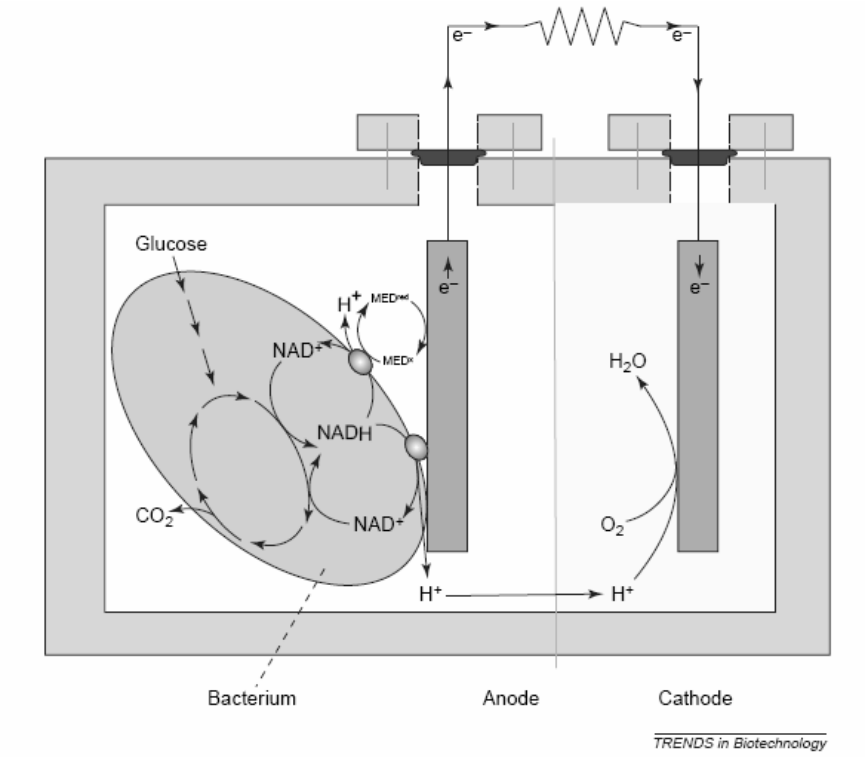


Figure 2-1. Diagram of a MFC (Rabaey and Verstraete 2005)

It has been known for almost a century that bacteria produce electricity (Potter 1911), but it has only been recently that extensive research has begun. There have been three generations of microbial fuel cells (Ieropoulos et al. 2005):

- Gen-I: use synthetic redox mediators
- Gen-II: use natural mediating properties of sulphate/sulphide
- Gen-III: no external soluble mediators (mediatorless)

The first mediatorless MFC was demonstrated in 1999 by Kim et al. Mediatorless MFCs show the most promise so far with five-fold higher power production and conversion efficiency of 95% (Ieropoulos et al. 2005). In less than a decade, power production by mediatorless MFCs has increased by several orders of magnitude. (Logan and Regan 2006)

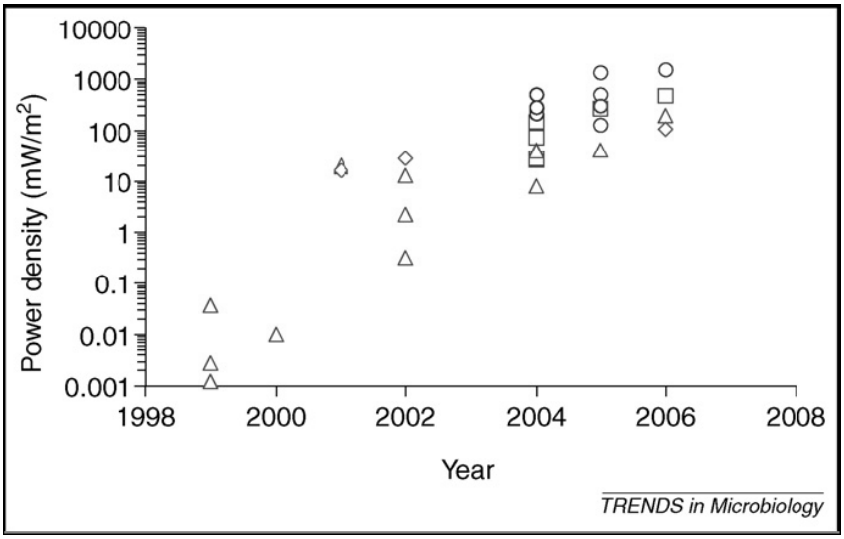


Figure 2-2. "Power production for mediatorless MFCs on the basis of published results. Power production continues to be limited by systems that have the cathode immersed in water [aqueous cathodes (triangles) and sediment MFCs (diamonds)]. Substantial power production has been possible using air-cathode designs in which the cathode is exposed to air on one side and water on the other (squares). In general, wastewaters have produced less power than systems using pure chemicals (glucose, acetate and cysteine in the examples shown; circles)" (Logan and Regan 2006)

Microorganisms in MFCs

Bacteria which are useful in microbial fuel cell operation have the ability to transfer electrons to an electrode (anode), instead of their characteristic electron acceptor (Rabaey et al. 2004) and are classified as electrochemically active.

A diverse set of microorganisms have been discovered to be electrochemically active, see Table 2-1 for a summary. A majority of these are proteobacteria (Phung et al. 2004), and some of the most common are iron reducing organisms, making them also capable of transferring electrons to an electrode. Microorganisms in the family *Geobacteraceae* are some of the most predominant in highly anoxic conditions (Lovley 2006), including species such as: *Geobacter metallireducens*, (Bond et al. 2002), *Geobacter sulfurreducens* (Bond and Lovley 2003), and the psychrotolerant *Geopsychrobacter electrodiphilus* (Holmes et al. 2004). Other iron reducing bacteria include the *Desulfuromonas acetoxidans* (Bond et al. 2002) and *Rhodoferax ferrireducens* from marine sediments (Chaudhuri and Lovley 2003), as well as newly discovered species phylogenetically related to *Clostridium butyricum* (Park et al. 2001), and another related to *Aeromonas hydrophila* (Pham et al. 2003).

While iron-reducing bacteria are predominant, many other species have also been found to be electrochemically active. *Geothrix fermentans* was the first isolate outside of the *Proteobacteria* found to completely oxidize organic compounds linked to electrode reduction (Bond and Lovley 2005). *Geopsychrobacter electrodiphilus* was enriched and isolated with Fe(III) oxide, grown with an electrode serving as the sole electron acceptor and transferred approximately 90% of the electrons available. It grows at temperatures between 4 and 30°C, with an optimum temperature of 22°C (Holmes et al. 2004).

Many of the electrochemically active bacteria remain to be identified. Some of these species have been used in pure culture to generate electricity in MFCs. However, they have relatively low energy transfer efficiency compared to mixed microbial communities endogenous to wastewater, marine sediments, and livestock manures (Rabaey et al. 2003). MFCs that make use of mixed bacterial cultures have some important advantages over MFCs driven by pure cultures: higher resistance to process disturbances, higher substrate consumption rates, lower substrate specificity, and higher power output (Rabaey et al. 2004). These microbial communities allow the electrochemically active bacteria to take advantage of the hydrolysis, fermentation, and anaerobic oxidation performed by other species to provide readily degradable substrates, making the general food web in MFCs similar to methanogenesis in all but the final step. The combined activity of fermentative microorganisms coupled with the oxidation of fermentation products by *Geobacteraceae* appears to be a more competitive process (Lovley 2006).

Highly effective microbial communities can be obtained by repeatedly harvesting the bacteria from the anode, leading to a consortium with columbic efficiency of 80%. (Rabaey et al. 2004). *Escherichia coli* have even been shown capable of electrochemically-evolving in fuel cell environments through a natural selection process (Zhang et al. 2006). The type of MFC also determines the nature of the microbial community. Batch systems allow for the production of soluble electron shuttles while continuous operation necessitates some form of direct contact (Lovley 2006).

Metabolism of MFC Microorganisms

As stated before, bacteria gain energy for metabolism and reproduction by transferring electrons and protons from a reduced substrate at a lower potential to an

electron acceptor at a higher potential. For example, with acetate, electricity production occurs following reactions:

Anode: $CH_3COO^- + 4\ H_20 \rightarrow 2\ HCO_3^- + 9\ H^+ + 8\ e^-$ Equation 2-1

Cathode: $O_2 + 4\ H^+ + 4\ e^- \rightarrow 2\ H_20$ Equation 2-2

Table 2-1. Electrochemically active bacteria

Microorganism	**Electron Donor**	**Transfer mechanism**	**Reference**
Alcaligenes faecalis	Not reported	shuttle	Rabaey et al. 2004
Enterococcus gallinarum	Not reported	shuttle	Rabaey et al. 2004
Shewanella putrefaciens	Not reported	shuttle	Angenet et al. 2004
P. aeruginosa, Pseudomonas spp	Not reported	shuttle	Rabaey et al. 2004
Shewanella oneidensis MR-1	Not reported	nanowires	Gorby et al. 2006
Synechocystis PCC6803	Not reported	nanowires	Gorby et al. 2006
Pelotomaculum thermopropionicum	Not reported	nanowires	Gorby et al. 2006
Geobacter metallireducens	Not reported	Not reported	Bond et al. 2002
Geobacter sulfurreducens	acetate and hydrogen	contact, nanowires	Bond and Lovley 2003 Reguera 2006
Geopsychrobacter electrodiphilus	acetate, organic acids, amino acids, long-chain fatty acids, and aromatic compounds	Not reported	Holmes et al 2004
Rhodoferax ferrireducens	glucose and other sugars	Not reported	Chaudhuri and Lovley 2003
Geothrix fermentans	Acetate, lactate, malate, propionate, and succinate	shuttle	Bond and Lovley 2005
Brevibacillus agri	Not reported	Not reported	Aelterman et al. 2006
Clostridium butyricum	Not reported	Not reported	Park 2001
Aeromonas hydrophila	glucose, glycerol, pyruvate and hydrogen	Not reported	Pham 2003

The potential energy available from these reactions can be represented visually with the electron tower (Figure 2-3). The larger the difference between the reduction potentials, the more energy is released. The total energy released during a reaction can be calculated as:

$$\Delta G = - nF\Delta E \qquad \text{Equation 2-3}$$

Where: ΔG = total energy

n = the number of electrons exchanged

F = Faraday's constant (96,485 $J*V^{-1}*mol^{-1}$)

ΔE = difference between the reduction potentials of the electron acceptor and donor

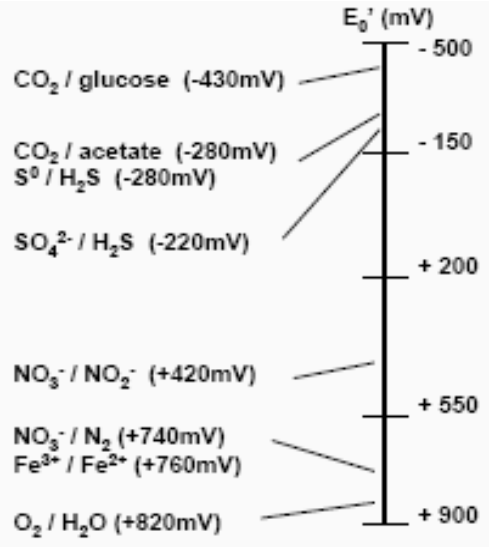


Figure 2-3. Electron Tower. Modified from *Brock Biology of Microorganisms*, 2003

Using this equation, the theoretical potential difference of a MFC when electrons are transferred from glucose to oxygen is approximately 1.2 V (ΔE = (+0.82V) – (-0.43V) Logan et al. 2006), leading to an energy gain of 200 kJ/mol of

glucose (2 electrons per molecule NADH). However, the measured MFC voltage is considerably lower due to a number of losses. These losses can be categorized as follows: (*i*) ohmic losses, which include resistance in the cation exchange membrane, electrodes, interconnections, and compartment solutions; (*ii*) bacterial metabolic losses; (*iii*) mass transport or concentration losses of different species; and (*iv*) activation losses, which occur because of the energy needed to transfer the electrons in an oxidation/reduction reaction. These losses combined are the internal resistance (R_{int}) of the MFC. The actual maximum power of the system varies inversely with the total resistance of the system squared. While the external resistance can be varied, R_{int} is fixed, and therefore limiting. (Logan et al. 2006). Design efforts to reduce R_{int} are ongoing.

Bacteria can use both respiration and fermentation during electrical production. During respiration, the substrate is oxidized with subsequent liberation of protons and electrons. All electrons, not captured for growth, within the bacteria can theoretically be transported to the electron acceptor. Fermentative metabolic pathways are used by some microorganisms when no readily available electron acceptors are present in the bacterial environment. During fermentation, bacteria will redeposit the liberated electrons on the oxidized substrate. The fermentation products are further oxidized at low anode potential by anaerobic bacteria such as Geobacter species, capable of removing electrons from acetate under MFC conditions (Bond and Lovley 2003).

It has been shown that generation of electrical current from a MFC was inhibited by various inhibitors of the respiratory chain. (Kim et al. 2004) Processes using oxidative phosphorylation have regularly been observed in MFCs, yielding high energy efficiencies (Rabaey et al. 2003). Approximately 2/3 of the electrons remain in the produced fermentation products such as acetate, leaving a maximum of 1/3 which can be used to generate current (Logan 2004).

Once the electrons have been liberated, they need a method of transport to reach the electrode. This extracellular electron transfer has been found to occur through three main pathways: direct contact of membrane-bound proteins, extracellular electron shuttle, or bacterial nanowires.

Electrodes in MFCs can act as an electron acceptor through direct contact with the bacteria as shown by the species *Geobacter sulfurreducens.* The electrode-attached cells were shown to completely oxidize acetate while producing electricity (Bond and Lovley 2003). Other bacteria that use this pathway are *Aeromonas hydrophila* (Pham et al. 2003) and *Rhodoferax ferrireducens* (Chaudhuri and Lovley 2003). Direct electron transfer by cell to cell contact is important as well (Stams et al. 2006).

A more common method of electron transfer for bacteria is through the use of soluble electron shuttles. To work efficiently, the shuttles must possess a redox potential (E_0) that is positive enough compared to the biological electron carrier (e.g. reduced cytochromes or NADH) to extract electrons, but negative enough compared to the anode electrode, to be oxidized at its surface (Ieropoulos et al. 2005). Soluble compounds, like humic substances, quinones, phenazines, and riboflavin, can function as extacellular electron mediators (Stams et al. 2006, Rabaey et al. 2005). Several species, (Table 2-1), including *Geothrix fermentans* (Bond and Lovley 2005), produce a compound that promotes electrode reduction. Inactivation of the genes responsible for mediator production in a *Pseudomonas aeruginosa* MFC isolate reduced the current generation by a factor of 20. It has also been found that the redox mediators produced by one bacterium can also be used by other bacterial species to reach the electrode (Rabaey et al. 2005).

The third method for extracellular electron transfer is through nanowires. A nanowire is an electrically conductive pilus-like appendage, see Figure 2-4 (Gorby et

al. 2006). Several bacterial species that have nanowires are listed in Table 2-1. For example, *Shewanella oneidensis* MR-1 produced nanowires in direct response to soluble electron-acceptor limitation, and nanowires produced by the oxygenic phototrophic cyanobacterium *Synechocystis* PCC6803 and the thermophilic, fermentative bacterium *Pelotomaculum thermopropionicum* reveal that electrically conductive appendages are not exclusive to metal-reducing bacteria and may, in fact, represent a common bacterial strategy for efficient electron transfer and energy distribution (Gorby et al. 2006). Nanowires allow cells at a distance from the anode to remain viable, and there is no decrease in the efficiency of current production as the thickness of the biofilm increases. An electronic network of nanowires permeating the biofilm can promote long-range electrical transfer (Reguera et al. 2006). There is also evidence that nanowires are important in interspecies electron transfer (Logan and Regan, 2006).

MFC Substrates

MFCs have been operated using a wide variety of substrates (see Table 2-2): glucose, acetate, butyrate (Liu et al. 2005a), cysteine (Logan et al. 2005), proteins, (Heilmann and Logan 2005), lignocellulose (Rismani-Yazdi et al. 2006), as well as complex substrates such as domestic wastewater (Cheng et al. 2006), swine manure slurry (Min et al. 2005), landfill leachate (Frew and Christy 2006), meatpacking wastewater (Heilmann and Logan 2005), and seafloor sediments (Tender et al. 2002). MFCs were also capable of converting dissolved sulfide to elemental sulfur, which implies a recovery of energy otherwise lost in a methane digester. (Rabaey et al. 2006). This may also lead to a decrease in the hydrogen sulfide produced during anaerobic digestion.

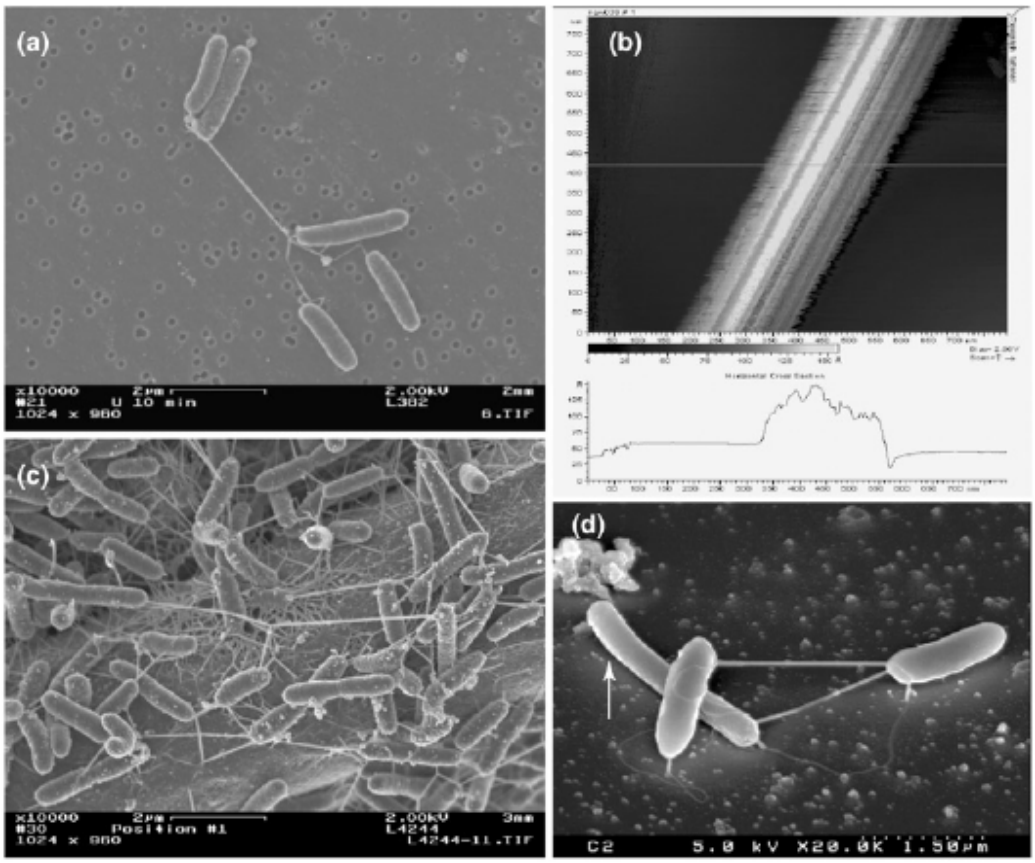


Figure 2-4. "(a) A scanning electron micrograph (SEM) of wild-type *Shewanella oneidensis* MR-1 grown under electron-acceptor-limited conditions, showing pilus-type nanowires that connect to other cells. (b) STM image of a single pilus-type nanowire from wild-type MR-1 (lateral diameter of 100 nm, topographic height of 5–10 nm) showing ridges and troughs running along the long axis of the structures consistent with a bundle of wires. The corresponding conductivity of the pilus as the tip moves over the indicated surface is shown beneath the STM image. (c) The anode from an MFC colonized by *S. oneidensis* MR-1. (d) An SEM image of *Pelotomaculum thermopropionicum* and *Methanothermobacter thermautotrophicus* (arrow) in methanogenic co-cultures showing pili connecting the two genera. Subsequent STM imaging has shown that the pili are conductive." (Logan and Regan, 2006)

Table 2-2. MFC substrates

Substrate	Power (mW/m^2)	Coulombic efficiency	Overall energy recovery	Current density (A/m^2)	Reference
Glucose (2 g/L-d)	3600	75%	65%	NA	Rabaey et al. 2003
Acetate (800 mg/L)	506	10-31%	3-7%	2.2	Liu et al. 2005
Butyrate (1000 mg/L)	305	8-15%	2-5%	0.77	Liu et al. 2005
Bovine serum albumin (1100 mg/L)	354	20.6%	NA	1.1	Heilmann and Logan 2005
Peptone (300 mg/L)	269	6%	NA	0.85	Heilmann and Logan 2005
Meat Packing wastewater (6010 mg/L COD)	139	15%	NA	1.15	Heilmann and Logan 2005
Swine wastewater	261	10%	NA	1.4	Min et al. 2005b

MFC System Parameters

The most significant block to achieving high power densities in MFCs is the system architecture, not the composition of the bacterial community (Logan and Regan 2006). That is why much of the current research is devoted to determining the optimal operating parameters and system setup of a microbial fuel cell.

Several suggestions were developed to ensure effective startup of a MFC. Start up is most successful when the biofilm is harvested from the anode of an existing active MFC, while enrichment with anaerobic sludge led to low power density. Also placing a second electrode (on a separate circuit) into the MFC in order to culture electrochemically active bacteria was found to not work successfully because the cathode size could not support the additional reactions. Enrichment of iron-reducing

bacteria can be achieved by using a soluble ferric iron medium and a ferric oxide-coated electrode (Kim et al. 2005).

Other parameters that have optimized performance include pH, temperature, and ionic strength. The highest current currently reported was generated at pH 7 (Gil et al 2003), while decreasing the temperature from 32 to 20 °C reduced power output by only 9% primarily as a result of the reduction of the cathode potential, and increasing ionic strength from 100 to 400 mM by adding NaCl increased power by reducing internal resistance (Liu et al. 2005a). Coulombic efficiency can be improved by adding 2-bromoethanesulfonate to inhibit methanogenic bacteria (Kim et al. 2005).

Anode

To be the preferred electron acceptor, the anode should be available with a higher (more positive) potential than other possible substrates in the waste stream, such as sulphate or iron, so that the energetic gain will be much higher for bacteria that can deliver to the anode. (Logan and Regan 2006). If however the anode potential is too low, electricity production will cease and fermentation processes will start.

Several methods have been shown to increase anode performance beyond the standard graphite electrode: from bioengineering a reconstituted glucose oxidase monolayer (Katz et al. 1999) to bound electron mediators including Mn^{4+}-graphite and neutral red covalently linked woven graphite anodes (Park and Zeikus 2003). Several combinations of materials were also tested with between 1.5- and 2.2-fold greater kinetic activity than plain graphite: graphite modified by adsorption of anthraquinone-1,6-disulfonic acid or 1,4-naphthoquinone, a graphite-ceramic composite containing Mn^{2+} and Ni^{2+}, and graphite modified with a graphite paste containing Fe_3O_4 or Fe_3O_4 and Ni^{2+} (Lowry et al. 2006). Another successful anode is based on tungsten carbide that presently allows current densities of up to 3 mA/cm^2 (Rosenbaum et al. 2006). It

remains to be seen if the improvements in performance of these anodes can outweigh the increased cost of production.

Cathode

The cathode is an important factor in the performance of a MFC due to the poor kinetics of oxygen reduction reaction in a neutral pH medium (Cheng et al. 2006b). Other physical and chemical environmental effects also influence the thermodynamics and the kinetics of the electrocatalytic oxygen reduction (Zhao et al. 2006). There are two general options for a cathode, either a chamber filled with with some form of dissolved electron acceptor or a chamberless cathode that is exposed directly to oxygen in the air.

Some compounds can be used as the final cathodic electron acceptor in a cathode chamber. Higher cell voltages can be achieved than dissolved or atmospheric oxygen cathodes (ferricyanide: 361 mV; MnO_2: 470 mV) because the concentrations of the compounds are much higher, leading to more favorable kinetics and a higher realized cathode potential. For example, biomineralized manganese oxides, deposited by *Leptothrix discophora*, provide a current density almost 2 orders of magnitude higher than oxygen (Rhoads et al. 2005). Ferricyanide has also been used, and achieved the one of the highest power densities in a MFC of 4310 mW/m^2 by decreasing the internal resistance to only 3 Ω (Rabaey et al. 2003). However, power generation with ferricyanide or MnO_2 is not sustainable. Ferricyanide must be externally regenerated, and soluble manganese can be lost over time. (Logan and Regan 2006)

Some MFCs have used dissolved oxygen in water (Bond et al. 2002, Tender et al. 2002, Logan et al. 2005) as the electron acceptor. This works well when the MFC is in an aerated solution such as seawater (Tender et al. 2002). However, the cost of

artificially aerating the cathode chamber is prohibitive when scaling up. Therefore, open air cathodes have become the more favored option (Min and Logan 2004, Oh et al. 2004).

Platinum is usually used as a catalyst with oxygen and is held on the electrode with a binder such as Nafion (perfluorosulfonic acid) or polytetrafluoroethylene (PTFE). An optimum of four layers of PTFE and platinum was found to increase the cathode potential (increase of 117 mV) and a 171% increase in the Coulombic efficiency (from 19.1% to 32%), a 42% increase in the maximum power density (from 538 to 766 mW/m^2), and measurable water loss was prevented over a standard commercially available cathode. However, Nafion performed slightly better as a Pt binder than PTFE (Cheng et al. 2006b)

Research has shown that the density of platinum loading can be greatly reduced compared with those required for hydrogen fuel cells, with only slightly reduced performance (cathode potential reduced from 20 to 40 mV and maximum power density was reduced an average of 19% when Pt loadings were decreased from 2 to 0.1 mg/cm^2) (Cheng et al. 2006b). Platinum can be reduced because the cathode is not the rate limiting reaction in the configuration used in these studies (Zhao et al. 2006).

Less expensive, non-precious metal catalysts have also been researched to replace platinum. Examples include engineering of a layered bioelectrocatalytic cathode of cytochrome *c*:cytochrome oxidase couple (Katz et al. 1999), pyrolyzed FeIII phthalocyanine (Rosenbaum et al. 2006, Zhao et al. 2006), and cobalt tetramethoxyphenylporphyrin (CoTMPP) (Zhao et al. 2006). Further research on replacing the Pt catalyst with CoTMPP, produced slightly improved performance above 0.6 mA/cm^2, but reduced performance (<40 mV) at lower current densities by an average of 12% (Cheng et al. 2006b). Another possibility is the use of biocathodes

that use bacterial metabolism to accept electrons from the cathode (He and Angenent 2006). This research shows that platinum can be replaced by cobalt– and iron–organic mixture catalysts with little reduction in performance, although the longevity of such materials is not well studied.

Changing the relative size of the cathode has also improved performance in some cases. Tripling the surface area of the cathode increased power density by 22%. A further increase in the cathode area by a factor of three increased the voltage by only 11% (Oh et al. 2004).

Proton/Cation Exchange Membrane

A proton (cation) exchange membrane (PEM/CEM) can be used to separate the cathode and anode liquids into different chambers, or just to act as a barrier that keeps materials other than protons from reaching the cathode (Logan and Regan 2006). It selectively only allows protons to pass through. Most MFC studies thus far applied Nafion (Dupont) proton exchange membranes (PEMs). However, Nafion membranes are sensitive to (bio)fouling by ammonium. The best overall results have been obtained using an Ultrex (Membranes International) cation exchange membrane (Rabaey et al. 2004)

Increasing the size of the PEM has been shown to improve MFC performance. For a fixed anode and cathode surface area, power density increased with the PEM size in the order 45 mW/m^2 (A_{PEM}=3.5 cm^2), 68 mW/m^2 (A_{PEM}=6.2 cm^2), and 190 mW/m^2 (A_{PEM}=30.6 cm^2). PEM surface area was shown to limit power output when the surface area of the PEM was smaller than that of the electrodes due to an increase in internal resistance (Oh and Logan 2006).

Besides the increase in internal resistance, the PEM also has a drawback of creating a pH gradient. During the course of fuel cell operation, a significant pH

gradient may build up between the cathode and the anode, which may lead to a decrease of microbial activity at the anode and the decrease of the oxygen reduction performance at the cathode. (Zhao et al. 2006)

When Min et al. used a salt bridge (see Figure 2-5A) in place of a PEM, the power output was greatly reduced. The low power output was directly attributed to the higher internal resistance of the salt bridge system: 19,920 ohm versus membrane system: 1286 ohm (Min et al 2005a).

Another study removed the proton exchange membrane completely. In a single chamber system, the power density increased with glucose from 262 mW/m^2 (40-55% Coulombic efficiency) to 494 mW/m^2 (9-12% Coulombic efficiency) and with wastewater from 28 mW/m^2 (28% Coulombic efficiency) to 146 mW/m^2 (20% Coulombic efficiency). The drop in Coulombic efficiency is do to an increase in oxygen flux from 0.05 mg/hr to 0.187 mg/hr allowing aerobic bacteria to scavenge any oxygen that may enter the chamber and degrade a portion of the substrate without electrical generation (Liu and Logan 2004). However, without a PEM, growth on the cathode and poisoning of the cathode catalyst can occur.

Even with a PEM, small amounts oxygen can enter the anode chamber. Several methods of removing this oxygen have been tested. Nitrogen gas sparging did not influence power production, but increased overall Coulombic efficiency (47 to 55%) versus without gas sparging (19%). L-cysteine (a chemical oxygen scavenger), increased power when put in a pure culture of *G. metallireducens*. Suspended cells (a biological oxygen scavenger) in mixed culture removed oxygen that diffuses through the membrane (Min et al 2005a).

MFC System Designs

Many different designs have emerged for MFCs (Figure 2-5 and Figure 2-6). A widely used and inexpensive design is a two chamber MFC built in an "H" shape (Figure 2-5F), consisting usually of two bottles connected by a tube containing a PEM. With this basic setup, either liquid or gas can be placed into either anode or cathode chamber. H-shape systems are acceptable for basic parameter research, such as examining power production using new materials, or types of microbial communities that arise during the degradation of specific substrates, but they typically produce low power densities (Logan et al. 2006).

Much larger power densities have been achieved using oxygen as the electron acceptor when aqueous-cathodes are replaced with air-cathodes (Logan et al. 2006). In the simplest configuration, called a single chamber MFC, the anode and cathode are placed on either side of a tube, with the anode sealed against a flat plate and the cathode exposed to air on one side, and the aqueous substrate on the other (Figure 2-5E).

Many advances on these basic designs have emerged in an effort to increase power density or provide for continuous flow through the anode chamber. Some basic improvements that can be made are decreasing the distance between the anode and cathode from 4 to 2 cm resulted in an increase in power generation from 720 to 1210 mW/m^2 from decreases in the internal resistance (Liu et al. 2005b). Similar results were seen with a miniature MFC where short diffusion lengths and high surface-area-to-chamber volume ratio enhanced power density (Ringeisen et al. 2006). Also successful is the use of advective flow through a porous anode toward the cathode with 1-cm electrode spacing. Using glucose, the maximum power was 1540 mW/m^2 with a Coulombic efficiency of 60% (Cheng et al. 2006a).

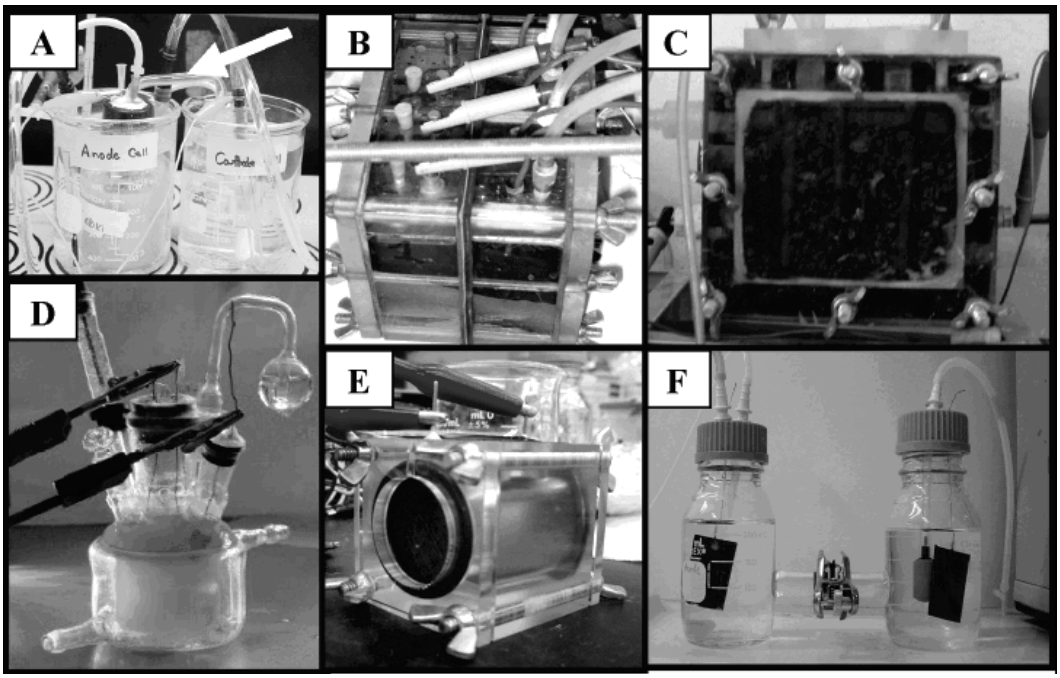


Figure 2-5. Types of MFCs used in studies: (A) two chamber system with a salt bridge (shown by arrow) (Min et al 2005a); (B) four continuous MFCs where the chambers are separated by the PEM (Rabaey et al. 2003); (C) same as B but continuous flow-through anode of granular graphite matrix and close anode-cathode placement (Rabaey, Ossieur et al. 2005); (D) photoheterotrophic MFC (Rosenbaum et al. 2005); (E) single chamber MFC with air cathode (Liu and Logan 2004); (F) two-chamber H-type system (Logan et al. 2005).

The highest power density to date was achieved using a design with four continuous MFCs side-by-side where the chambers are separated by the PEM (Figure 2-5B). Using a dissolved ferricyanide cathode chamber, a power density of up to 3.6 W/m^2, with Coulombic efficiency of 89% with a flow of 3 g $COD*L^{-1}*d^{-1}$ (Rabaey et al. 2003). When this design was modified to include a granular graphite matrix for the anode and close anode-cathode placement (Figure 2-5C), the power output reached a maximum of 49 W/m^3 (1 kg COD/m^3) with Coulombic and energy conversion efficiencies of 50.3% and 26.0%, respectively. (Rabaey, Ossieur et al. 2005).

Figure 2-5D is an example of a photobiological fuel cell that utilizes the metabolic activity of *Rhodobacter sphaeroides* for the generation of electricity based

on the in situ oxidation of photobiological hydrogen. It achieves energy conversion efficiency of 8.4% and a current density of 28.8 A/m^3 (Rosenbaum et al. 2005).

A tubular, single-chambered, continuous MFC with granular graphite matrix as the anode and a ferricyanide solution in the cathode chamber (Figure 2-6A) has achieved a maximum power density with acetate of 90 W/m^3 and Coulombic efficiency of 75% (loading rates: 1.1 kg COD/m^3 *day (Rabaey, Clauwaert, et al. 2005).

Another method to improve performance is to use a cathode inside the anode chamber. In a continuous, upflow MFC with an internal cathode (Figure 2-6B), volumetric power was 29.2 W/m^3 with soluble COD removal efficiencies exceeding 90% with an overall internal resistance of 17.13 ohms (He et al. 2006).

An additional design is a flat plate MFC (Figure 2-6C). Operated as plug flow reactor with domestic wastewater, the average power density was 72 mW/m^2 at a liquid flow rate of 0.39 mL/min (42% COD removal, 1.1 h HRT). When the HRT was extended to four hours, the COD removal increased to 79%, with a lower average power density of 43 mW/m^2. The maximum power density was achieved at a flow rate of 0.22 mL/min: power density of 63 mW/m^2 with a current of 1.03 mA and 326 ohm internal resistance (Min and Logan 2004).

A variation on the single chamber design used eight graphite electrodes and a single air cathode (Figure 2-6D). With continuous flow of domestic wastewater, power reached a maximum of 26 mW/m^2, 69 ohm internal resistance, COD removal of 80%, with a Coulombic efficiency of less than 12% (Liu et al. 2004).

Six individual continuous MFC units in a stacked configuration (Figure 2-6E) using graphite granules for the cathode and anode, produced a maximum averaged power output of 258 W/m^3 using a hexacyanoferrate cathode. When placed in series the voltage reached 2.02 V at 228 W/m^3 (CE: 12.4%), and when placed in parallel the

current reached 255 mA at 248 W/m^3 (CE: 77.8%). After combined operation, the initial microbial community decreased in diversity and gram positive species became dominant. Also, the power output of the individual MFCs tripled from 73 W/m^3 to 275 W/m^3, the mass transfer limitations decreased, and the MFC internal resistance was lowered from 6.5 to 3.9 ohm. The combined average current density and voltage was similar to the performance of individual MFCs. Therefore, stacked MFCs will not deliver higher power densities than the individual MFCs. Yet, they create the possibility to produce an averaged power at more practical voltages and currents, and increased kinetics of COE destruction on a volumetric basis (Aelterman et al. 2006).

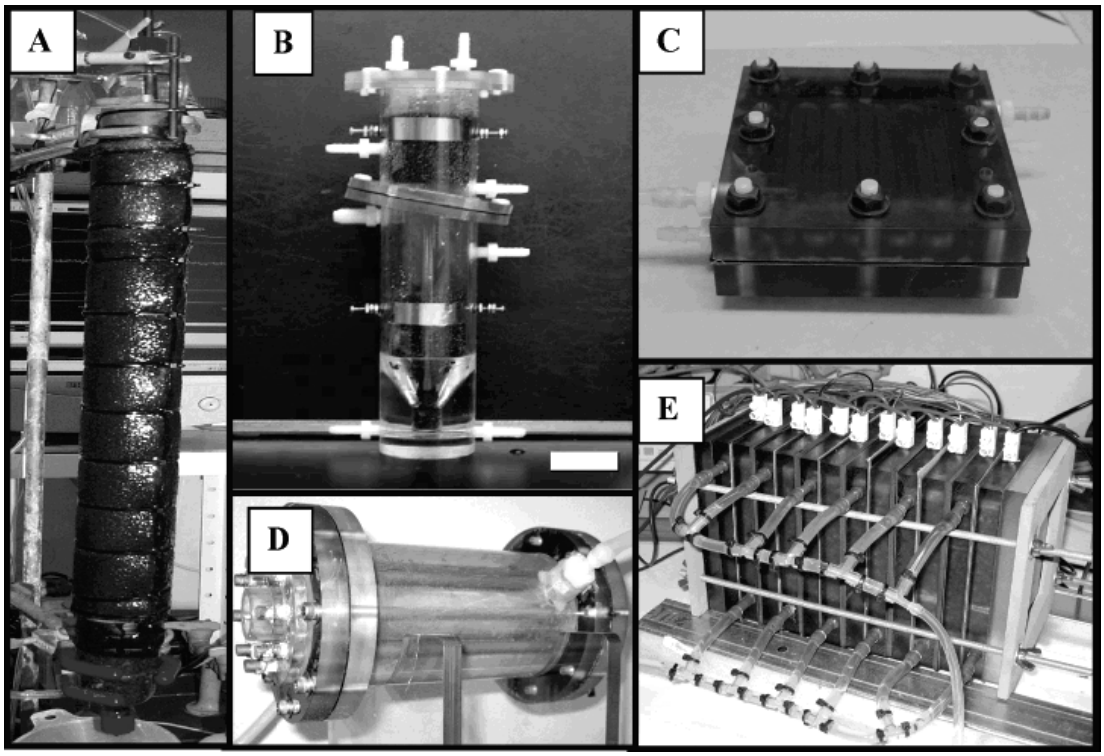


Figure 2-6. MFCs used for continuous operation: (A) upflow, tubular MFC with inner graphite bed anode and outer cathode (Rabaey, Clauwaert, et al. 2005); (B) upflow, tubular MFC with anode below and cathode above (He et al. 2005); (C) flat plate design where a channel is cut in the blocks so that liquid can flow in a serpentine pattern across the electrode (Min and Logan 2004); (D) single-chamber system with an inner concentric air cathode surrounded by a chamber containing graphite rods as anode (Liu et al. 2004); (E) stacked MFC, in which 6 separate MFCs are joined in one reactor block (Aelterman et al. 2006).

Scale up of Microbial Fuel Cells

Microbial fuel cells have many challenges to overcome before they are ready for scale up. One of the main challenges is the expense of the materials needed. The capital expenditure is in the order of $1.5 million per MW capacity installed, for energy production from fossil fuel by conventional combustion processes, wind turbines, anaerobic digestion and chemical fuel cells. Currently, MFCs are estimated to be at a level 10 times above that (Rabaey and Verstraete 2005), mainly because of the low power densities achieved thus far. Further development of non-precious metal catalysts and lower cost PEMs can lower this expense.

Another system constraint is the need for large surface area to support the electrochemically active bacterial biofilm. Some materials will not be suitable for scale-up because of their inherent lack of durability or structural strength (e.g. carbon paper), or cost (e.g. graphite rods) (Logan and Regan 2006).

Continuous operation will be necessary, and will need to meet several criteria. The system will need to bring in substrate, remove biodegradation byproducts, not become clogged or be easy to clean, and have enough turbulence to allow protons to move to cathode (Rabaey, Lissens, and Verstraete 2005). Also important will be the optimization of power production versus COD removal. Depending on the goals of the design, longer retention times will remove more organic substrates (COD), while shorter retention times will produce more power. Even when optimization is achieved, it remains to be seen whether MFCs will become economically viable (Angenent et al. 2004).

Anaerobic Digestion Overview

Anaerobic digestion (AD) is a process by which organic substrates are microbially degraded. The gases given off during this process, called biogas, consists

of methane (50%-80%), carbon dioxide (50%-20%), and trace levels of other gases such as carbon monoxide, ammonia, and hydrogen sulfide (EPA, 2000). Theoretically, biogas production occurs following the reaction:

$$C_6H_{12}O_6 \rightarrow 3\ CH_4 + 3\ CO_2 \qquad \text{Equation 2-4}$$

AD offers the advantages of high organic removal rates, low energy-input requirement, energy production (i.e. methane), and low sludge production (Angenent et al. 2004). The biogas can be used for energy production in a variety of ways: heat, electricity, pipeline natural gas, fuel for vehicles, or hydrogen production.

Microbiology of Anaerobic Digestion

Anaerobic bioconversion of complex organic material to methane requires four major steps and five physiologically distinct groups of microorganisms. A basic overview can be seen in Figure 2-7 while a more detailed diagram including major microbial species is shown in Figure 2-8. The large polymers are initially broken down though hydrolysis to produce simpler compounds that are further degraded by fermentation. The next step involves hydrogen-producing acetogenic bacteria growing in syntrophic associations with hydrogenotrophic methanogens, which keep the hydrogen partial pressure low enough to allow acetogenesis to become thermodynamically favorable (this process is referred to as interspecies hydrogen transfer) (Angenent et al. 2004). This interspecies hydrogen transfer between microorganisms is the driving force for complete biodegradation in methanogenic environments (Stams et al., 2006). Other methanogens then convert the acetate into further methane and carbon dioxide.

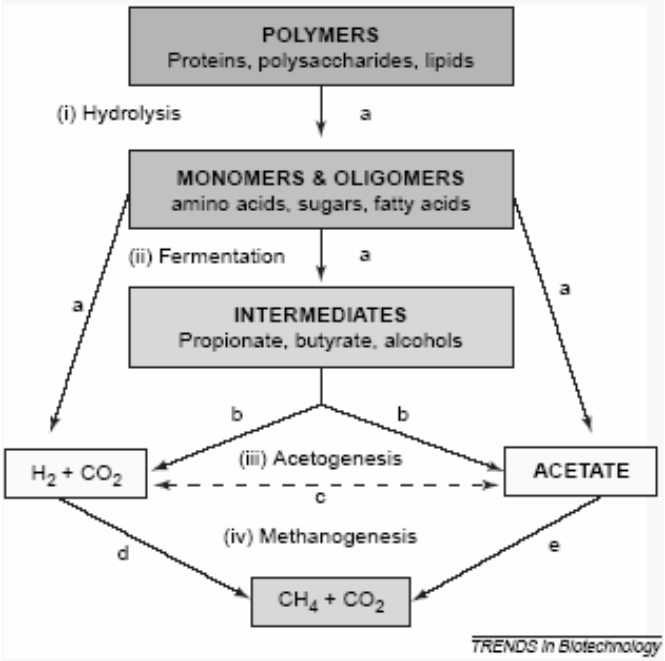


Figure 2-7. Microbial process of biogas production (Angenent et al. 2004)

Anaerobic Digestion System Designs

Several different designs of anaerobic digestion systems have been developed that operate under differing conditions (Table 2-3). The primary determining factor for the type of AD is the type of manure collection and storage system: whether it is liquid, slurry, semi-solid, or solid. Also, most systems in use today require meso (~38^{o}C) or thermophilic (~55^{o}C) temperatures to achieve sufficient turnover and limited methane solubility (Pham et al. 2006).

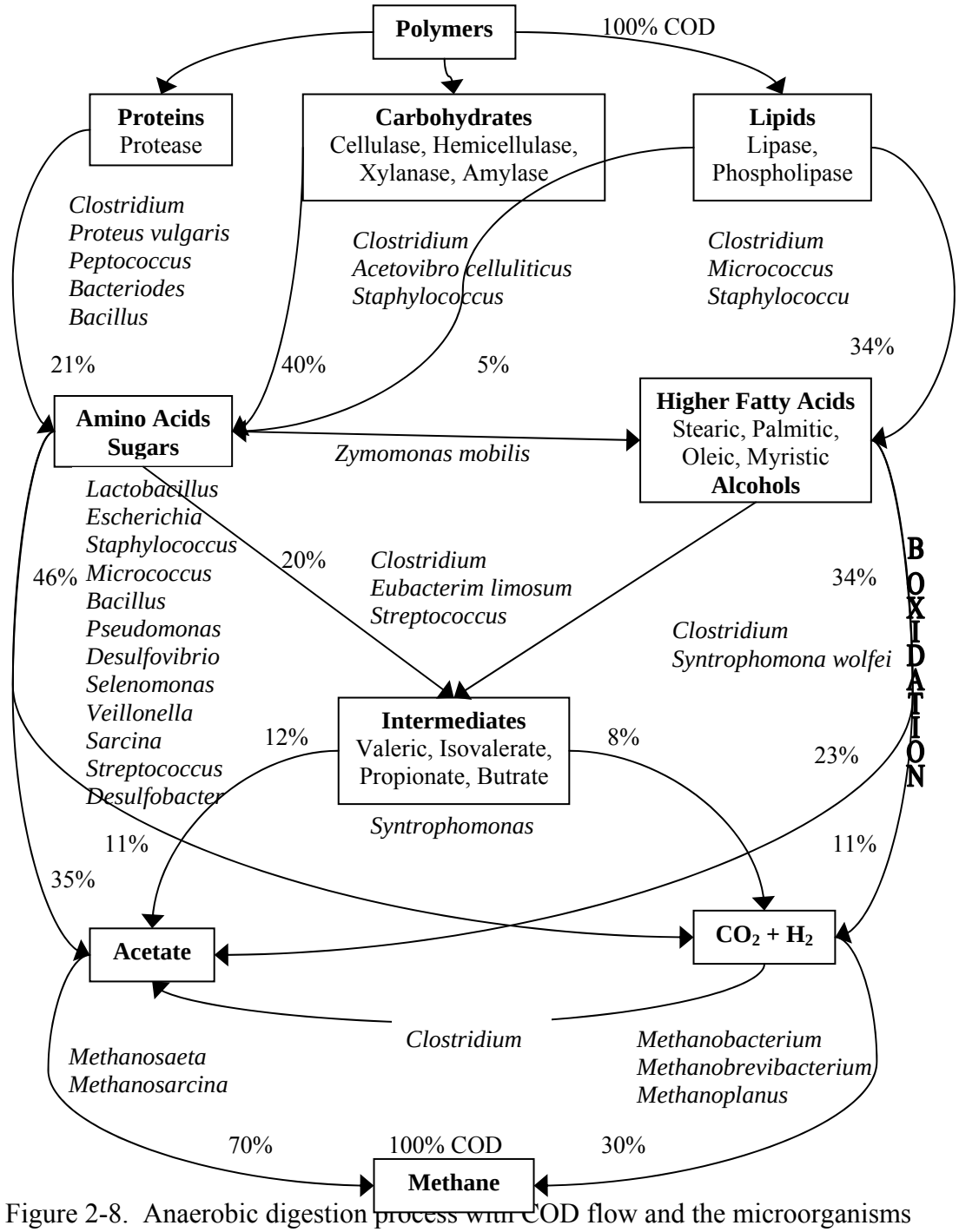


Figure 2-8. Anaerobic digestion process with COD flow and the microorganisms predominantly responsible for each step. (Gujer and Zehnder 1983)

The most basic of the designs is the covered lagoon (Figure 2-9). These systems typically operate in warmer climates, but some have also been installed in more temperate regions (Roos and Moser 2000). Anaerobic lagoons have been used for several decades and are designed as a longer term storage and treatment for low solids manure systems. They are the largest of the manure treatment options and usually consist of a lined storage pond with pipe inlets that is emptied periodically. A gas impervious cover can be installed on these lagoons allowing biogas capture (Gooch 2006). This design is typically used when a farm has an existing lagoon where odor control or energy production is desired.

Table 2-3. Characteristics of Typical Agricultural Anaerobic Digesters (Roos and Moser 2000)

	Covered Lagoon	**Complete Mix**	**Plug Flow**	**Fixed Film/USAB**
Vessel:	Deep lagoon	Round/Square In/Above ground Tank	In ground rectangular tank	Above ground tank
Level of Technology:	Low	Medium	Low	Medium
Additional Heat:	No	Yes	Yes	No
Total Solids:	0.5-1.5%	3-11%	11-13%	3%
HRT (days):	40-60	15+	15+	2-3
Farm Type:	Dairy/Hog	Dairy/Hog	Dairy only	Dairy, Hog
Optimum Climate:	Temperate/ Warm	All	All	Temperate/ Warm

Most of the original anaerobic digesters constructed on farms in the U.S. were plug flow digesters. Influent material is introduced at one end of the digester and is presumed to flow linearly, like a plug, through the digester and exits at a point of time in the future that equals the digester's hydraulic retention time (HRT) (Figure 2-10).

The designed HRT in most plug-flow digesters is about 21 days. The aspect ratio for plug-flow digesters normally ranges from 4 – 6 to 1. It is critical that the solids content of the influent be maintained around 12% to ensure proper flow and prevent solids partitioning (Gooch 2006).

Figure 2-9. A partially covered anaerobic lagoon (Gooch 2006)

Figure 2-10. A plug flow anaerobic digester from a 500-cow dairy (Gooch 2006).

Completely mixed digesters are tanks, above or below ground, that treat slurry manure with a solids concentration in the range of 3 to 10 percent (Figure 2-11).

These structures require less land than lagoons or plug flow digesters and are heated (Roos and Moser 2000). These systems are more complex than plug flow and have relatively higher equipment and operating costs (not necessarily overall costs) than plug flow systems (Gooch 2006).

Figure 2-11. A vertical mixed AD from an 800-cow dairy (Gooch 2006)

A fixed-film anaerobic digester is a digester that contains media within the treatment volume of the digestion vessel. The purpose of the media is to provide surface area for operative microbes to grow and propagate with the overall goal of reducing the HRT yet maintaining a reasonable level of biogas production (Gooch 2006). The upflow anaerobic sludge blanket [UASB] reactor has a similar goal of retaining the complex microbial consortium by using the formation of biological granules, which can efficiently convert wastewater organic compounds into methane in small 'high-rate' reactors. (Angenent et al. 2004). These digesters are much smaller than the previously discussed systems. However they are designed to operate with dilute waste streams to prevent clogging.

CHAPTER 3

MATERIALS AND METHODS

Dairy Manure

Dairy manure slurry was collected from AA Dairy (Candor, NY) and refrigerated at 4^{o}C until needed. The dairy has a scraped alley manure system, uses woodchip bedding, and does not include any other materials in the manure slurry. The manure is used as the experiment substrate without any changes other than dilution with deionized water when noted.

MFC Construction

The MFC consisted of an anode and cathode placed on opposite sides in a plastic (Plexiglas) cylindrical chamber 4 cm long by 3 cm in diameter based on the design by Liu and Logan, 2004 (empty bed volume of 21 mL; anode projected surface area per volume of 25 m^2/m^3) as illustrated in Figure 1 and Figure 2. The anode electrodes were made of Toray Carbon paper (density = 0.44 g/cc, porosity = 78%), while the cathodes were made of Carbon cloth coated at a rate of 0.5 mg/cm^2 with a paste of a binder and 10% Platinum (E-Tek). The anode was covered with a solid brass sheet to maintain anaerobic conditions at the anode and serve as a lead into the circuitry, while the cathode was open to the air. Wire was soldered to each of the brass leads and connected with a 470 ohm resistor. A proton exchange membrane was not used.

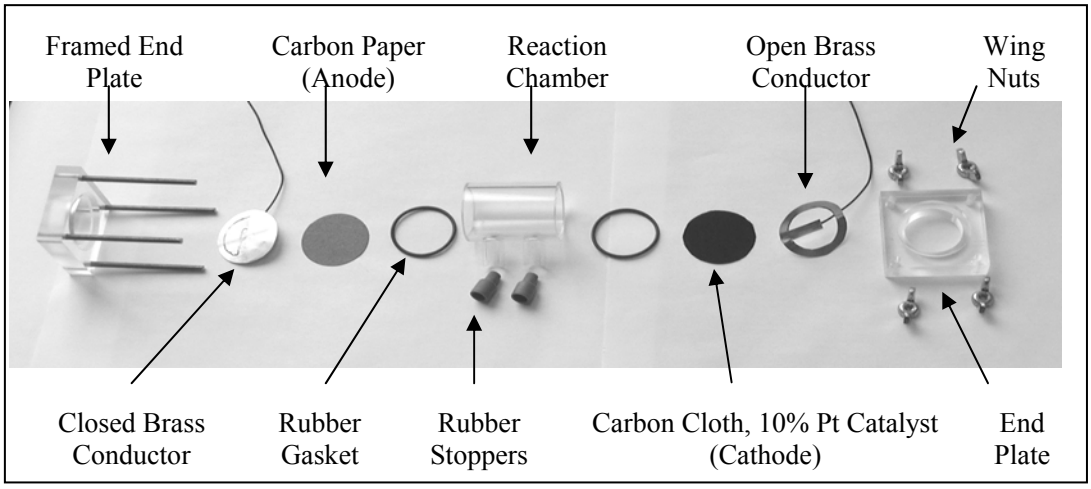


Figure 3-1. MFC construction

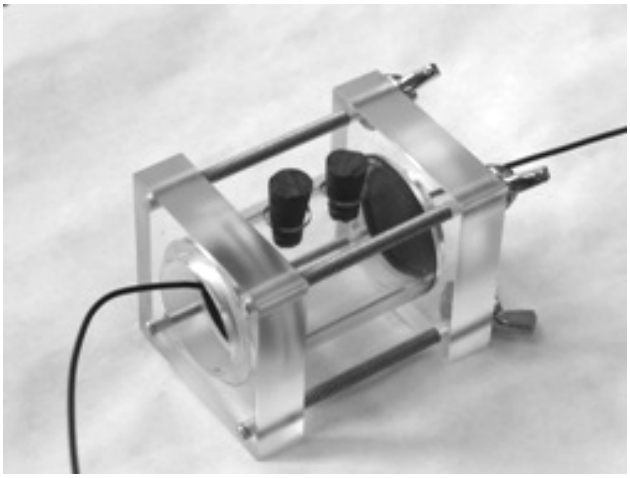


Figure 3-2. Assembled MFC

Electrochemical Measurement

Voltage (V) is recorded and used to calculate the power (P) and current (I) according to:

$$P = V^2/R \qquad \text{Equation 3-1}$$

$$I = V/R \qquad \text{Equation 3-2}$$

Power was normalized by the cross-sectional area (projected) of the anode, A = 7 cm^2, or as volumetric power with the wet volume of the MFC chamber = 21 cm^3. A

470 ohm resistor (R) is used in all MFC experiments. Data from the MFCs was collected continuously with a computerized data acquisition system using EasyData software (Weber-Shirk 2001).

Polarization curves were constructed for the MFCs using a potentiostat (Gamry Instruments, FAS2, Warminster, PA) in two electrode setup by changing the external resistance from open to short circuit with a scan rate of 100 mV/s. The working electrode was connected to the cathode and both the counter and reference electrodes were connected to the anode. "Polarization curves can generally be divided in three zones: (*i*) starting from the open circuit voltage at zero current, there is an initial steep decrease of the voltage: in this zone the activation losses are dominant; (*ii*) the voltage then falls more slowly and the voltage drop is fairly linear with current: in this zone the ohmic losses are dominant; (*iii*) there is a rapid fall of the voltage at higher currents: in this zone the concentration losses (mass transport effects) are dominant. In MFCs, linear polarization curves are most often encountered. For a linear polarization curve, the value of the internal resistance (R_{int}) of the MFC is equal to the slope," (Logan et al 2006):

$$R_{int} = -\Delta V / \Delta I \qquad \text{Equation 3-3}$$

A power curve that describes the power density as the function of the current density was calculated from the polarization curve. As no current flows for open circuit conditions, no power is produced. From this point onward, the power increases with current to a maximum. Beyond this point, the power drops due to the increasing ohmic losses and electrode overpotentials to the point where no more power is produced (short circuit conditions), (Logan et al 2006).

Biogas analysis

The biogas potential of the samples was determined using the method established by Owen et al. (1978). 20 mL of sample is placed in a 120 mL bottle (100 mL of headspace). The bottle was sparged with gaseous nitrogen and carbon dioxide (70% and 30% respectively) for two minutes, and then capped with a septum. A solid state pressure sensor with a range of 30 PSI (207 KPa, PX26-030GV, Omega) was attached to a needle and inserted in the septum. The bottle was then incubated at 37^{o}C for 45 days with continuous pressure monitoring.

Figure 3-3. Anaerobic digestion bottle with pressure transducer

To determine the volume of biogas produced, each bottle was first zeroed to account for any calibration errors. The average pressure from the duplicate water blanks was then subtracted to account for the expansion of the initial gases as well as variations in atmospheric pressure. The volume of the biogas was then calculated by using the ideal gas law:

$$PV = nRT \qquad \text{Equation 3-4}$$

$$V_1 - P_1V_1 / P_2 = V_{biogas}$$ Equation 3-5

Where P is the pressure and V is the volume of the gas, either initial or final, n is the number of moles of gas, R is the gas constant (8.314472 $m^3 \cdot Pa \cdot K^{-1} \cdot mol^{-1}$), and T is temperature. Since n, R, and T remain constant, they drop out of the equation.

The composition of the biogas was analyzed using a gas chromatograph (GC) (SRI 8610C, HAY SEP-D column) with a 1-m fused-silica column of 0.32 mm inner diameter, and Helium was used as a carrier gas. The GC was calibrated to measure the relative composition of nitrogen, carbon dioxide, and methane. Methane was detected using a flame ionization detector (FID), while nitrogen and carbon dioxide used a thermal conductivity detector (TCD). The composition of the water blank headspace gases were used to determine the initial volume of nitrogen and carbon dioxide present from sparging, so that the composition of the biogas produced during anaerobic digestion could be compared. See the appendix for full calculations.

The energy collected from anaerobic digestion was then calculated assuming the energy content of methane is 1,000 BTU/standard cubic foot, a 35% efficient conversion of methane to electricity, and using the determined methane content of the samples.

Chemical Analysis

Total COD was measured in duplicate at the beginning and end of each trial (HACH COD system 2002). A COD test vial has 0.2 mL of sample added and was heated at 150°C for two hours, after which the COD was determined using a spectrophotometer (HACH 2002). Total solids and total volatile solids of the stock manure solutions were measured in triplicate using Standard Methods (APHA AWWA WEF 1999): first the sample was dried for one hour at 105°C to determine total solids, and then ignited at 550°C for one hour to determine volatile solids.

The Coulombic efficiency (CE) is a common measure of performance of microbial fuel cells defined as the ratio of total Coulombs actually transferred to the anode from the anodic chamber, to maximum possible Coulombs if all substrate removal produced current (Logan et al. 2006). The total Coulombs obtained was determined by integrating the current over time, so that the Coulombic efficiency for an MFC run in fed-batch mode, evaluated over a period of time, was calculated as:

$$CE = M \Sigma(I\Delta T) / Fbv_{An}\Delta COD \qquad \text{Equation 3-6}$$

Where M = 32, the molecular weight of oxygen, I (C/s) is current during time step ΔT (sec), F is Faraday's constant = 96,400 C/mol, b = 4 is the number of electrons exchanged per mole of oxygen, v_{An} is the volume of liquid in the anode compartment (L), and ΔCOD is the change in COD (mg/L).

Methane production is generally calculated as the milliliters of methane produced gram of COD destroyed. However, this does not easily compare with the MFC output. Therefore, we use Coulombic efficiency as a measure of AD as well. The number of Coulombs captured during AD can be calculated by:

$$C_{methane} = n_{methane}\, b\, F \qquad \text{Equation 3-7}$$

Where $n_{methane}$ is the number of moles of methane produced, b = 8 is the number of electrons exchanged per mole of methane, and F is Faraday's constant = 96,400 C/mol. The equation for CE then becomes:

$$CE = M\, C_{methane} / Fbv_{An}\Delta COD \qquad \text{Equation 3-8}$$

Statistical Analysis

In biological systems and experiments testing these systems, large amounts of variation are often common. To clarify this variation, statistical methods were used. All tests were conducted using 5% level of significance. The Student's *t* test can be used to test the null hypothesis that the means of two normally distributed populations

are equal. Both one and two tailed tests were used. When more than two procedures were compared, analysis of variance (ANOVA) was used to determine if there is a significant difference between the means or if the observed spread is simply due to chance. Both of these tests were conducted using the Data Analysis tool in Microsoft Excel©.

Microbial Fuel Cell Operation

To start the MFCs, the chamber was filled with full strength dairy manure. When transferring manure, it was important not to disturb the biofilm on the anode. To accomplish this, the MFC was placed vertically with the anode on the bottom. The cathode end was removed, and the substrate liquid aspirated out of the chamber. Solids that settled to the bottom were removed with a spatula, being careful to minimally disturb the biofilm on the anode.

Since a PEM was not used, water was lost by evaporation through the cathode. Deionized water was added to the MFCs at the end of each test to enable accurate measurements of COD destruction.

Experimental Operation – Varying Manure Concentration

For the first set of experiments, the strength of the manure (as measured by COD) was varied by diluting the manure with deionized water. Four manure strengths: non-diluted, 1:10, 1:100, and 1:1000 dilutions as well as deionized water blank were tested. For each strength, three MFCs were filled with the same manure mixture and operated for two days at 20^{o}C in triplicate. The experiment proceeded from a 1:10 dilution down to the deionized blank, and finally the full strength manure.

Experimental Operation – Varying Operating Temperature

During the second set of experiments, temperature was varied. Manure was diluted 1:10 with deionized water (COD = 10,700 mg/L) and used to fill the MFCs in all trials. Trials were run for 4 days, first at 20°C and 37°C each in triplicate, then 55 °C in duplicate, and then finally back at 20°C. At elevated temperatures, water evaporated more quickly. Deionized, sparged water that was at incubator temperature was added daily to the MFCs during the 37°C and 55 °C trials. Also to prevent the anode from drying out, the MFCs were tilted at a 45 degree angle, with the cathode elevated.

Experimental Operation – MFC combined with AD

Combinations of MFCs and AD were examined. A stock solution of manure was used to fill five MFCs, five MFCs that did not have a closed circuit (for a control to determine the amount of COD destroyed by mechanisms other than the electrochemically active bacteria, labeled "Unconn"), four AD bottles, and three refrigerator AD controls (to determine how much four days in the refrigerator influenced biogas production). The concentration of the manure stock was estimated by setting a target pressure for the bottle that would not cause the septum to leak due to excessively high biogas pressure build-up (see the Appendix for full calculations). The target pressure was set to 0.5 atmosphere (50,663 Pa). Assuming 395 mL of methane are produced for every gram of biodegradable COD destroyed, it was determined that the manure stock solution should have approximately 5000 mg/L of COD. All MFCs were operated four days at 20°C, while AD bottles were operated 45 days at 37°C.

Five different scenarios were examined. First AD was operated alone to determine baseline biogas production (labeled "AD"). Second MFCs were operated alone to determine baseline MFC operation (labeled "MFC"). Then three combinations were examined: MFCs before AD (labeled "After MFC"), AD before MFC operation, and MFC to AD back to MFC. For the first combination, MFCs were operated for four days, the manure solutions were then sampled (~1mL) for COD, and placed into separate AD digestion bottles. For the second combination, AD bottles were operated for 45 days, the manure solutions were sampled (~1mL) for COD, and then placed into MFCs for four days. The final combination adds another four day MFC run at the end of the first combination to explore whether bioelectricity production could still occur.

CHAPTER 4

RESULTS AND DISCUSSION

The first stage of this project was to determine if dairy manure was compatible with microbial fuel cell use. While previous data suggested it was feasible, explicit tests have not been reported. Non-diluted dairy manure was placed in a newly constructed MFC (Figure 4-1), and replaced at hour 117 and 166 by removing all of the manure except that closest to the anode to promote biofilm growth. Within a few hours electricity was being generated, and following the manure replacements, voltage output increased. These initial experiments demonstrated that electricity could be generated using dairy manure, and that the bacteria needed were endogenous in the manure slurry.

Figure 4-2 shows a 200 hour trial of an MFC using diluted manure with a COD of approximately 1000 mg/L. The shape of the curve shows a typical voltage response to manure input. Maximum voltage is usually reached within the first 24 hours, probably due to rapid consumption of readily biodegradable substrates. The voltage then slowly decreases as the electrochemically active bacteria must wait for substrates to be broken down through fermentation by other microbes in the microbial community. Eventually the MFC reaches it baseline voltage – the equilibrium voltage that the MFC can maintain without any exogenous substrates available in solution. Here, the only available substrates for growth and maintenance are provided by the death of other microbes. Even at this low COD concentration, it takes many days to completely degrade the available substrates, as determined by when voltage returns to baseline. However, most of the power is generated in the first few days, due to the high concentration of easily biodegradable substrates. Therefore in later experiments,

the MFC operation time was limited to 2 or 4 days. All of the MFC voltage curves can be found in the Appendix.

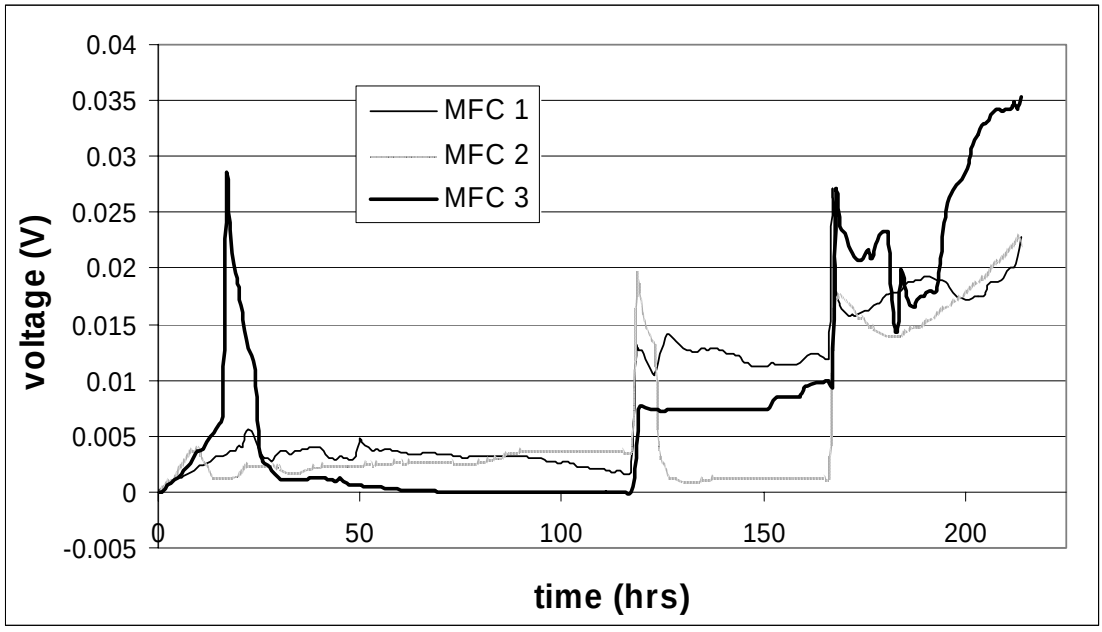


Figure 4-1. Startup, 20°C with replacements of full strength dairy manure at 0, 117 and 166 hours.

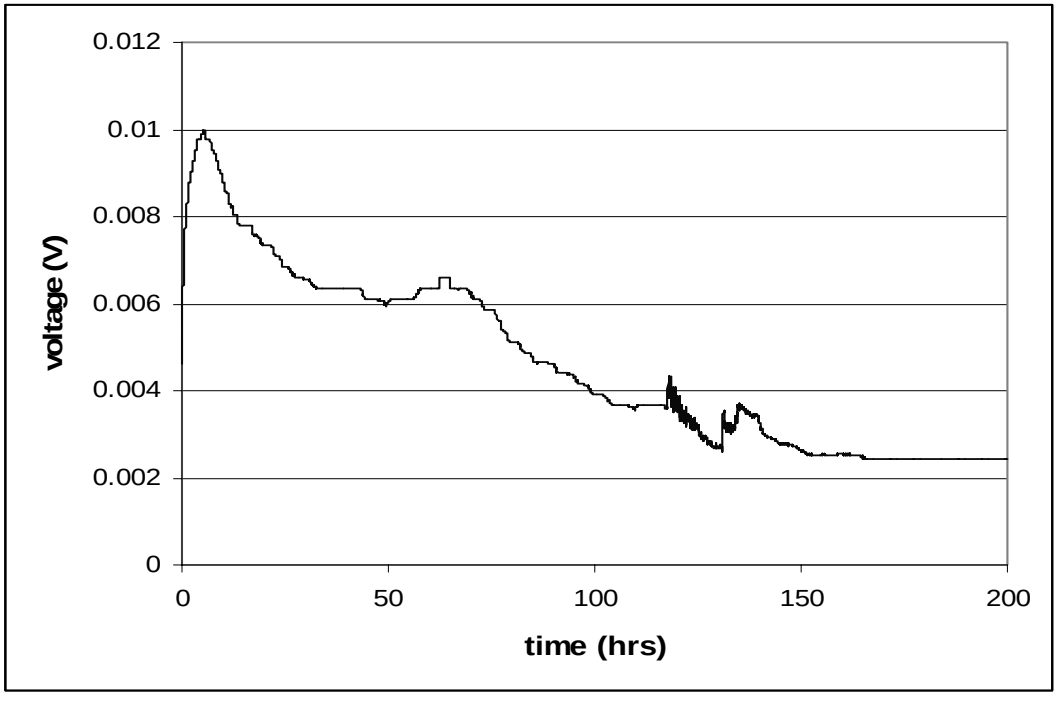


Figure 4-2. MFC operation, 20°C, 200 hours

Polarization curves (Figure 4-3), were obtained for three of the MFCs using a potentiostat. There were differences between individual MFCs, indicating there is likely variability in the microbial communities of each microbial fuel cell. The maximum power densities found for MFC 1, 2, and 3 were: 165, 101, and 143 mW/m^2, with internal resistances of 118 ohm, 108 ohm, and 102 ohm respectively. This internal resistance is the likely the limiting factor in the performance of the MFCs used in these experiments. A similar design used by Min et al. (2005) had an internal resistance of approximately 40 ohm, while their two chamber system had a higher internal resistance of 1800 ohm.

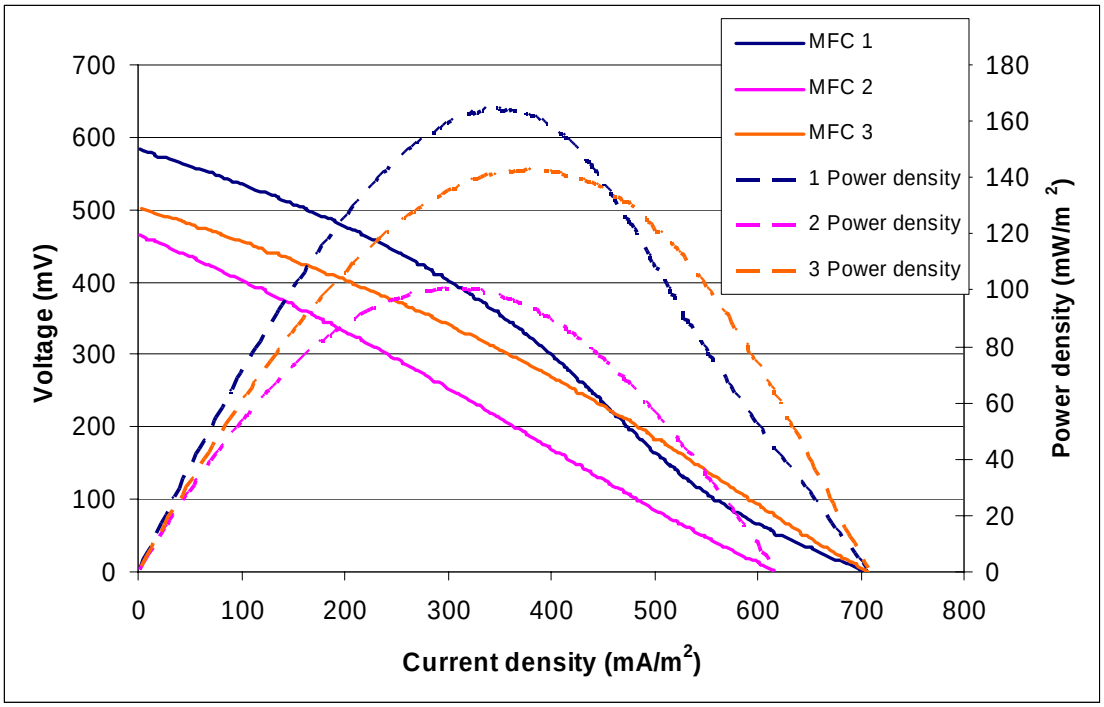


Figure 4-3. Polarization curves

Varying Microbial Fuel Cell input manure concentration

The first set of experiments measured power generation as a function of the input manure concentration to determine the affect of the concentration of organic matter (as measured by chemical oxygen demand, COD) on power production. Each MFC was operated with raw manure concentrations from 0.1 to 100 g/mL COD for two days each (20 ^{o}C) in triplicate. The maximum power density generated was 138 ± 19 mW/m^2 with undiluted manure. This is similar to the 182 mW/m^2 generated by the same MFC configuration using swine wastewater (Min et al. 2005), with the variation likely due to differences in resistor size, substrate, and microbial community.

Both average total Coulombs captured (Figure 4-4) and average maximum power density (Figure 4-5) were proportional to the strength of the manure solution (COD). This is inconsistent with other studies where a saturation-type relationship was found (Min et al. 2005 and Liu et al. 2005b). This suggests that the MFC output in our experiment was limited by the amount of readily biodegradable substrates present in the slurry, rather than reaction kinetics. Dairy manure has approximately 3% of the COD as volatile fatty acids (Knowlton et al. 2006). These include acetic (~50%) and butyric acids which are easily degraded in MFCs.

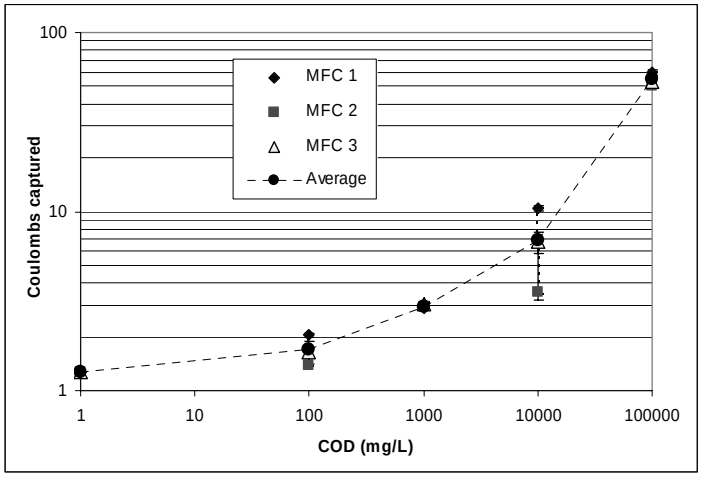


Figure 4-4. Coulombs captured by MFCs as a function of manure strength.

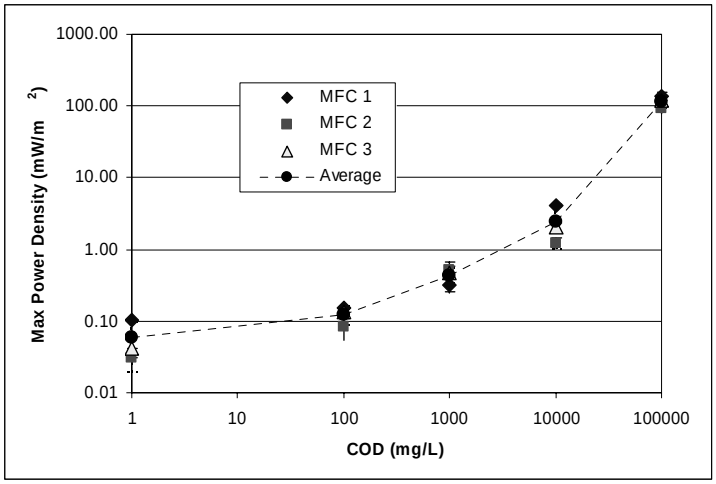


Figure 4-5. Maximum Power Density of the MFCs as a function of manure strength.

Varying Microbial Fuel Cell temperature

Power generation was next measured as a function of the MFC operating temperature to determine the affect on MFC performance at temperatures used in AD (37 ^{o}C for mesophillic, and 55 ^{o}C for thermophillic) compared to the lower operating temperature of 20 ^{o}C. Even though the highest power output was obtained using full strength manure, the manure was diluted 1:10 for ease of handling, making it easier to ensure complete emptying of MFCs between trials. The averaged triplicate data for the four day operation at each temperature are summarized in Table 4-1 through

Table **4-3**. Operation at 20 ^{o}C resulted in an average of 46% less energy capture (Coulombs), 32% lower maximum power density, and 22% lower average total power than when operated at 37 ^{o}C. The COD destruction remains nearly the same at both temperatures, most likely due to aerobic breakdown from oxygen leaking through the cathode. The coulombic efficiency was increased by mesophillic operation, because more of the COD destruction was captured as bioelectricity from the MFCs. However, when the fuel cells were operated at thermophillic temperature (

Table **4-3**), their performance fell below either that of the previous temperatures, and after they were returned to 20 °C, very little power was produced. This implies that the operation at thermophillic temperature inhibited/killed the electrochemically active bacteria.

A challenge of elevated temperature operation was the increase in water loss through evaporation. Without a proton exchange membrane (PEM), water vapor evaporated quickly out of the MFC chamber because of the permeability of the cathode. As the liquid level decreased, less surface area was available on the cathode, possibly limiting performance. At 20 °C the fuel cells lost approximately 3mL of water by the end of four days, this increases to about 3mL per day at 37 °C, and 5 mL per day at 55 °C. At the elevated temperatures, sparged, deionized water was added to make up for the evaporated losses. If scaled up, MFCs would likely be operated as a continuous flow through system rather than batch, causing evaporative losses to be less of a concern.

Table 4-1. Effect of temperature on MFC performance, three replicates of each MFC at each temperature (Operation time = 88.44 hrs).

Temperature = 37°C						
	Total coulombs	St Dev	Max Power Density (mW/m^2)	St Dev	Total Energy Captured (J)	St Dev
1	41.162	3.417	26.580	7.471	2.602	0.462
2	37.826	3.929	20.654	8.810	2.155	0.450
3	42.565	1.807	29.951	4.982	2.764	0.268
Average	40.518	2.434	25.728	4.706	2.507	0.315
Temperature = 20°C						
	Total coulombs	St Dev	Max Power Density (mW/m^2)	St Dev	Total Energy Captured(J)	St Dev
1	18.527	2.532	9.598	4.681	0.572	0.091
2	20.251	2.313	7.816	4.597	0.630	0.146
3	17.615	1.131	7.527	5.497	0.484	0.086
Average	18.798	1.339	8.313	1.121	0.562	0.073

Table 4-2. Chemical Oxygen Demand Removal and Coulombic efficiency, three replicates of each MFC at each temperature

Temperature	**37 °C**			**20 °C**		
	COD removal	St. Dev.	Coulombic eff.	COD removal	St. Dev.	Coulombic eff.
MFC1	34%	11	4.5	25%	4	2.7
MFC2	24%	6	5.8	16%	8	4.8
MFC3	22%	11	7.2	23%	12	2.8
Overall	27%	10	5.8	21%	9	3.4

Table 4-3. Thermophillic operation results (Operation time = 90 hours)

	55 °C		**20 °C after 55 °C**	
	Replicate 1	Replicate 2		
Total Coulombs				
MFC 1	9.394	4.377	MFC 1	2.770
MFC 2	9.331	4.340	MFC 2	2.950
MFC 3	7.156	4.206	MFC 3	2.965
MFC 4	6.686	4.875	MFC 4	3.498
Average	8.142	4.450	Average	3.046
St. Dev	1.423	0.293	St. Dev	0.314
Max Power Density				
MFC 1	1.766	0.995	MFC 1	0.096
MFC 2	1.605	0.860	MFC 2	0.137
MFC 3	1.416	1.072	MFC 3	0.116
MFC 4	1.830	0.696	MFC 4	0.148
Average	1.654	0.906	Average	0.124
St. Dev	0.185	0.165	St. Dev	0.023
Total Energy Captured (J)				
MFC 1	0.138	0.036	MFC 1	0.011
MFC 2	0.133	0.035	MFC 2	0.013
MFC 3	0.080	0.033	MFC 3	0.013
MFC 4	0.083	0.038	MFC 4	0.018
Average	0.108	0.036	Average	0.014
St. Dev	0.027	0.002	St. Dev	0.003

Microbial Fuel Cells combined with Anaerobic Digestion

The next set of experiments combined microbial fuel cells in series with anaerobic digestion. A stock manure solution was prepared with COD of 3.3 g/L,

0.6% total solids, and 81% volatile solids. This solution was used to fill the anaerobic digestion bottle reactors and MFCs. Anaerobic Digestion (labeled as AD replicates 1-4) was performed alone to establish the biogas potential of the manure which resulted in an average of 28.4 mL, as seen in Figure 4-6 and Table 4-4. For a check on the effect of four more days in the refrigerator before addition to bottles, three AD bottles were filled with the stock solution (labeled as Refrig replicates 1-3) after four days in the refrigerator. The refrigerator controls were shown to be statistically equivalent to the original AD bottles with P value of 0.315 (t-stat = -1.116, two tailed). Therefore, there was no statistical change in the biogas production from manure after it was in the refrigerator for four days.

To determine the effect of MFC operation on biogas production during anaerobic digestion, MFCs were operated for four days before the manure was moved to AD bottles (labeled After MFC 1-5). The average amount of biogas produced remained statistically equivalent with a mean of 21.7 mL. However, this is right on the edge of a 95% confidence interval with a P value of only 0.062 (t-stat = 2.293, two tail). This is mostly caused by "After MFC 2", which is much lower than the other trials.

Table 4-4. Total milliliters of biogas at the end of 45 day anaerobic digestion at 37°C (* malfunctioning pressure sensor, no data)

	AD	Refrig	Unconn	After MFC
1	22.849	29.777	21.897	22.114
2	26.697	29.119	*	16.194
3	27.604	26.064	23.974	25.095
4	28.373		21.729	*
5			26.066	21.722
Average	26.381	28.320	23.416	21.281
St. Dev.	2.452	1.982	2.040	3.711

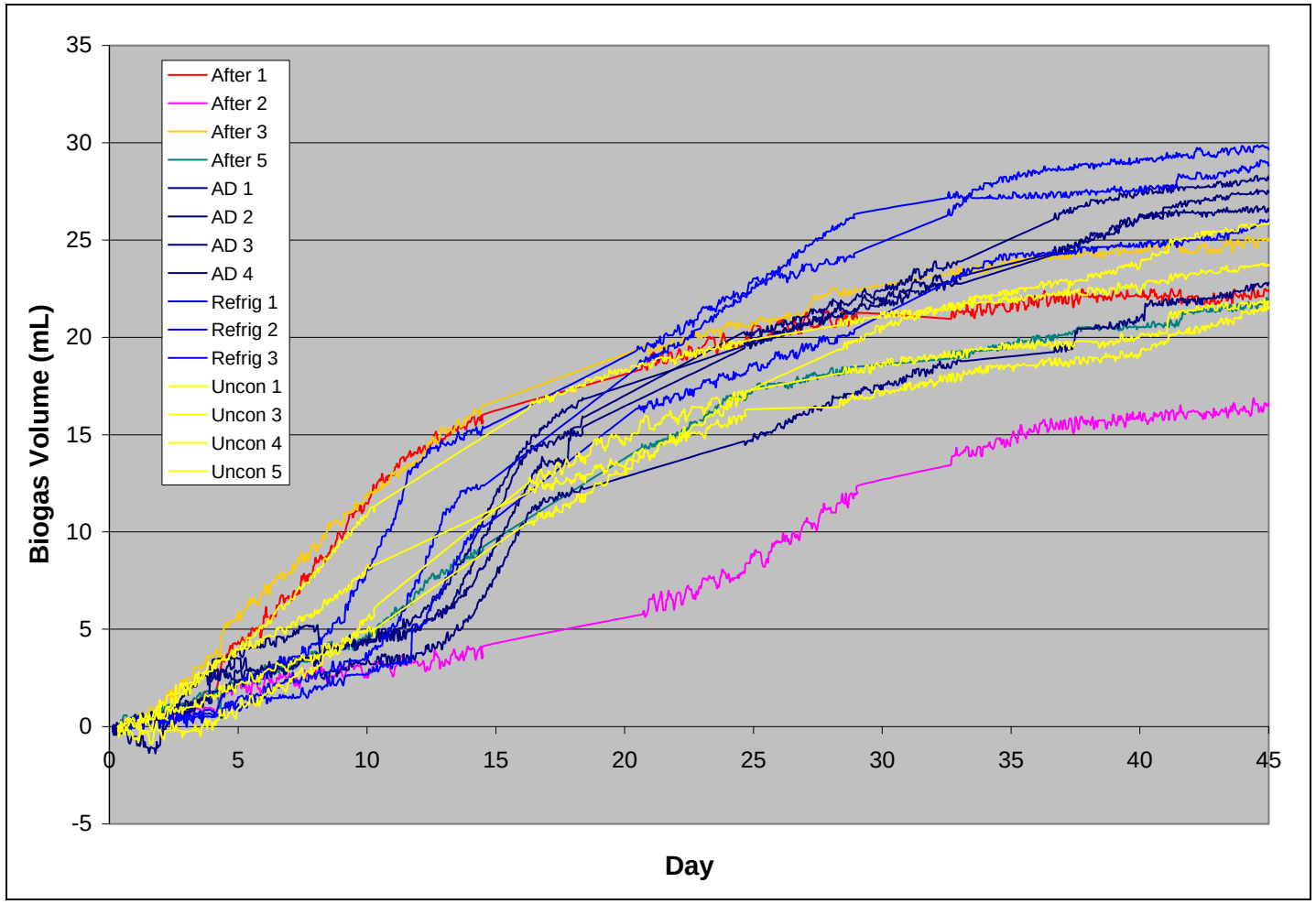


Figure 4-6. Biogas volume as a function of time during AD bottle trials. "After X" and "Unconn X" curves are manure which was in MFC X for four days before AD with electricity collection or unconnected respectively. "AD X" are replicates of manure that went straight into AD. "Refrig X" are replicates of manure that was in the refrigerator for four days before AD.

It was also important to determine if allowing the manure to sit in a MFC, even without electrical generation, affected the biogas production from AD. This was tested by placing the manure stock into the MFCs for four days, but leaving the electrode leads unconnected (labeled Unconn 1-5). These trials demonstrated that there is not a statistically distinguishable difference between the amount of biogas produced from AD alone (P value = 0.112, t-stat = 1.859, two tail) or MFC operation before AD (P value = 0.352, t-stat = 1.008, two tail), and leaving the manure sitting in an unconnected MFC for four days at 20^{o}C. However, when analysis of variance is used to compare all four data sets, the result suggests the means are not the same. (F stat = 4.778, P value = 0.023).

The composition of manure biogas is usually 55-60% methane, our values agree, with an average of 59% methane (Table 4-5). "After 2" had a similar biogas composition to the water blanks suggesting methanogenesis did not occur. However, "Unconn 2" had a shift in the composition, with very little carbon dioxide remaining.

Table 4-5. Biogas Composition (average and standard deviation excludes zeros.)

Trial	% Methane	Average/ St. Dev.
AD 1	62.25%	
AD 2	71.91%	64.49%
AD 3	63.27%	0.050678
AD 4	60.55%	
Refrig 1	59.28%	58.31%
Refrig 2	58.59%	0.011364
Refrig 3	57.06%	
After 1	54.63%	
After 2	57.64%	56.76%
After 3	58.04%	0.015214
After 4	0.00%	
After 5	56.74%	
Uncon 1	56.36%	
Uncon 2	0.00%	56.09%
Uncon 3	56.99%	0.007961
Uncon 4	55.89%	
Uncon 5	55.10%	

To further investigate the impact of microbial fuel cell operation on anaerobic digestion, the maximum slope of the biogas curves was compared between the different trials (Figure 4-6 and Table 4-6). Assuming that the composition of the biogas does not change significantly at the end of the run, the steep increase in biogas indicates peak methane production. Even with just visual inspection of the curves (Figure 4-6), it is clear that the AD alone bottles (AD and Refrig), have a much sharper rise in biogas production than the bottles that spent four days in a MFC (After MFC and Unconn). This relationship is shown in the statistical relationships. The t-test suggests that the AD and Refrig samples have statistically similar means with a P value of 0.115 (t-stat = -2.691, two tail), and that the After MFC and Unconn have the same means with a P value of 0.442 (t-stat = 0.835, two tail). These two groups have distinctly differing means of 2.9 and 1.3, as shown by the very low P value of 1.85E-5 (t-stat = 6.344).

Table 4-6. Maximum slope of biogas curves in milliliters per day (* no data)

	AD	Refrig	After MFC	Unconn
1	2.52	3.88	1.55	1.07
2	2.42	3.21	2.10	*
3	2.53	2.96	1.31	1.74
4	2.82		*	0.97
5			0.83	0.92
Average	2.573	3.350	1.448	1.175
St. Dev.	0.149	0.390	0.460	0.329

Another comparison between the trials is the timing of biogas production. Visual inspection of Figure 4-6 shows that biogas production appears to begin sooner in the AD bottles whose manure was first placed in a MFC for four days, both connected and unconnected. To capture the early differences in biogas production, a threshold of five milliliters was chosen, and the time to reach that threshold recorded (Table 4-7). Again the AD and Refrig samples have statistically indistinguishable

means (P value = 0.188, two tail, t-stat = 1.700) as well as the After MFC and Unconn samples (P value = 0.659, two tail, t-stat = 0.476). However, there is a lot of variation between the MFC samples, particularly After MFC 2, which starts very slowly. If After MFC 2 is not included in the t-test, these two groups (first in a MFC versus no pretreatment) are again statistically different with a low probability of the hypothesis of equal means being true (P value = 0.005, t-stat = 3.622). On an individual basis, these data show that in some of the samples, the four days in a MFC seem to have jump-started biogas production. This again seems to attest to the differences in microbial community. A MFC with a more active population of fermenter microbes will have more substrates available for methanogen conversion to biogas. The data suggests that MFCs 3, 4, and possibly 1 have more active communities while MFC 2 and 5 do not seem to shorten the timing of biogas production. Another piece of information that appears to support this hypothesis is the fairly strong correlation (0.698) of the timing of the biogas production between the bottles with manure that had been pretreated in the same MFC (i.e. After MFC 1 compared to Unconn 1). It is also important to note that when the time to reach 5mL is shortened, it is by more than four days, suggesting that the difference is not simply from sitting in the MFCs for those four days. A final point about the biogas timing is that the delay in biogas production from the AD only bottles is greater than what was expected, and led to the lengthening of the AD portion of the study to 45 days. The other factors of the AD bottles (COD, VS, total biogas production) were in the expected ranges, so it seems that the delay in biogas production for several months was likely caused by the storage of the manure in the refrigerator which may have led to inactivation of some of the microbial population. More time was therefore needed for the populations of the microbes to reach thresholds critical for biogas production.

Table 4-7. Time in days required to produce 5 mL of biogas (* no data)

	AD	Refrig	After MFC	Unconn
1	13.67	8.67	5.54	9.67
2	11.21	10.84	17.5	*
3	11.88	11.76	4.46	5.63
4	12.09		*	6.51
5			10.38	10.04

To look at the MFC performance before and after AD, four MFCs were filled with manure stock solution and operated for four days at 20^{o}C to establish MFC performance (Table 4-8, MFC). The average total coulombs captured when freshly diluted manure was added to the MFCs was 16.0 C, however there was a lot of variation between individual MFCs, with a standard deviation of 7.5 C. This variation is consistent with that in other trials showing the differences in performance of individual MFCs. Comparing these trials with the previous experiments, the Coulombs captured average is in the same range, being higher than the COD variation experiment, but lower then the 20^{o}C temperature experiment after adjustments for time and COD concentration are taken into account.

Table 4-8. Results of MFC operation (Operation time = 91.9 hours)

	MFC		**MFC after AD**		**MFC after MFC - AD**	
	Coulombs	Accum Energy (J)	Coulombs	Accum Energy (J)	Coulombs	Accum Energy (J)
1	14.074	0.302	6.192	0.056	3.163	0.016
2	8.702	0.116	6.070	0.054	4.869	0.034
3	28.220	1.217	6.706	0.066	5.043	0.037
4	17.173	0.471	3.505	0.019	2.456	0.009
5	11.950	0.217			7.508	0.081
Ave.	16.024	0.464	5.618	0.049	4.608	0.035
St. dev	7.484	0.440	1.436	0.021	1.961	0.028

To determine the performance of the MFCs after anaerobic digestion, five fuel cells were operated with the manure transferred after a 45 day anaerobic digestion.

There was a significant decrease in the MFC performance (Table 4-8, MFC after AD): average Coulombs captured decreased 65% from 16.0 to 5.6 C.

A final comparison can be drawn with the MFCs operated again after the manure had been both through the MFC and then anaerobic digestion. During this experiment the MFCs were operating not much above baseline, which is consistant with the negligible COD destruction observed during this same trial (Table 4-9, COD removal). Here the average Coulombs captured decreased a further 13% compared to the MFCs operated after AD alone and decreased 72% compared to the pre AD MFCs.

Looking at the system as a whole, there is a high correlation (0.84) between the performance of the MFC (measured as Coulombs captured) and the amount of biogas produced in the AD bottle following that MFC. This again suggests that some of the MFCs have microbial communities that, for unknown reasons, improve overall biogas production, as well as shortening the timing of that production. This could be a measure of how active the fermenter population of the MFC is. If the fermenters are better able to convert complex to simpler substrates, such as acetate or hydrogen, the more readily it can be converted into either bioelectricity in the MFCs or methane in the AD bottles. The sample "After MFC 2" was much lower than the other trials, and this could be linked to the type of microbial community present. As a MFC, it produced less power compared to the others (Table 4-8), had much lower biogas production when placed in an AD bottle (Figure 4-6) and then the AD after the time unconnected did not produce any methane (Table 4-5) and had a decrease in headspace carbon dioxide.

Another important aspect of manure management is reduction of the chemical oxygen demand to reduce potential hydrological impacts. The COD removal is recorded in Table 4-9. While there are large variations in the data, generally most of the COD destruction took place in the first process, either MFC or AD, that the

manure was subjected too. In order to more accurately compare these processes, the number of electron equivalents (eeq) generated was determined for each trial (Table 4-9). Anaerobic digestion consistently generated at least an order of magnitude more eeqs. However, the AD did operate for 45 days, while the MFC were only operated for four.

In a MFC, Coulombic efficiency (CE) is used to measure how many of the electron equivalents (eeqs) of degraded biomass (ΔCOD) is captured as electricity. Coulombic efficiency is inversely related to substrate concentration, this relationship is thought to be due to the effect of oxygen transfer into the system through the cathode. The higher the substrate concentration the longer the period of time needed to fully degrade the substrate. As the time period increases, more oxygen can leak into the system causing aerobic removal of the substrate, and lowering the overall CE. Substrate can also be removed using alternate electron acceptors, for example through sulfate reduction, heterotrophic denitrification, and methanogenesis (Min et al. 2005). In all MFC trials, this value was quite low (Table 4-9). Results from MFCs researched by others also have a wide range of CEs, however these CEs are on the lower end of the range. Factors that may contribute to the low CEs are the substrate, competing microbial physiologies, and design. Dairy manure is a highly heterogeneous mixture, including a large fraction of recalcitrant particles. COD measures all organic matter, whether it is bioavailable or not, biological oxygen demand (BOD) may be a better measure of the changes made by the MFC or AD. BOD tests have there own drawbacks that were not very compatible with this study. For example, they require large sample volumes and the BOD still may not reflect the portion of the organic matter that is available to anaerobes in a MFC. The design of the MFC in this study also negatively impacted CE. Eliminating the proton exchange membrane (PEM), decreases the cost and internal resistance of the MFC allowing increased power

densities. However, removing the PEM allows oxygen to easily diffuse into the MFC chamber. Using the value obtained by Liu and Logan (2004), 0.187 mg/hr, it can be estimated that after 4 days of operation approximately 18 mg of oxygen has diffused into the anode chamber. This oxygen is scavenged by aerobes, which can in turn degrade some of the organic substrates. Therefore a portion of the COD destruction is from microaerophilic breakdown, not bioelectricity production, decreasing the CE. From our estimate above, aerobic breakdown would account for approximately 860 mg/L of the COD removal, accounting for a significant amount of the total COD destruction.

Coulombic efficiency can also serve as a means to calculate the fraction of electrons stripped from the organic matter that were captured as methane. This is a different method of looking at methane data than is usually presented. A more common representation of methane production is given by the milliliters of methane produced per gram of COD destruction. Using the theoretical 8 eeq per mole of methane, a CE can be calculated. The values from this experiment were consistently over 100% (or above the theoretical maximum 395 mL/g COD) indicating that our COD destruction was underestimated. However, it is clear that AD is a much more efficient method to capture the electrons released during microbial biodegradation.

A final comparison of the MFCs and AD is presented in Figure 4-7 and Figure 4-8. The total energy recovery of each combination is graphed over time. The energy added through MFC generation (<1 J) is relatively small when compared to the 120 – 220 J from anaerobic digestion.

Table 4-9. COD removal, Coulombic efficiency, and energy capture from anaerobic digestion and MFCs. (* not calculable)

Trial	% COD removal	Coulomb efficiency %	Electron equivalents as CH_4 or electricity	mL of CH_4 / g COD
Unconn 1	47			
Unconn 2	39			
Unconn 3	44			
Unconn 4	47			
Unconn 5	28			
Unconn 1 after AD	39	93	5.17E-03	279
Unconn 2 after AD	36	*	*	*
Unconn 3 after AD	34	93	5.54E-03	352
Unconn 4 after AD	21	155	5.32E-03	509
Unconn 5 after AD	9	235	6.01E-03	1475
MFC 1	38	2	1.46E-04	
MFC 2	8	7	9.03E-05	
MFC 3	46	4	2.93E-04	
MFC 4	68	1	1.24E-04	
MFC 5	40	3	1.78E-04	
MFC - AD 1	35	146	5.07E-03	184
MFC - AD 2	37	89	4.76E-03	190
MFC - AD 3	7	1017	5.88E-03	252
MFC - AD 4	*	*	*	*
MFC - AD 5	20	282	5.29E-03	208
MFC - AD - MFC 1	*	*	3.45E-05	
MFC - AD - MFC 2	*	*	5.35E-05	
MFC - AD - MFC 3	*	*	5.54E-05	
MFC - AD - MFC 4	*	*	2.72E-05	
MFC - AD - MFC 5	26	4	8.29E-05	
AD 1	34	119	6.35E-03	337
AD 2	25	157	6.20E-03	532
AD 3	11	407	6.90E-03	1284
AD 4	31	150	7.46E-03	449
AD - MFC 1	43	1	6.44E-05	
AD - MFC 2	22	1	6.31E-05	
AD - MFC 3	36	1	6.97E-05	
AD - MFC 4	28	0	3.64E-05	
Refrig after AD 1	30	142	6.71E-03	496
Refrig after AD 2	15	339	7.924E-03	879
Refrig after AD 3	23	158	5.82E-03	559

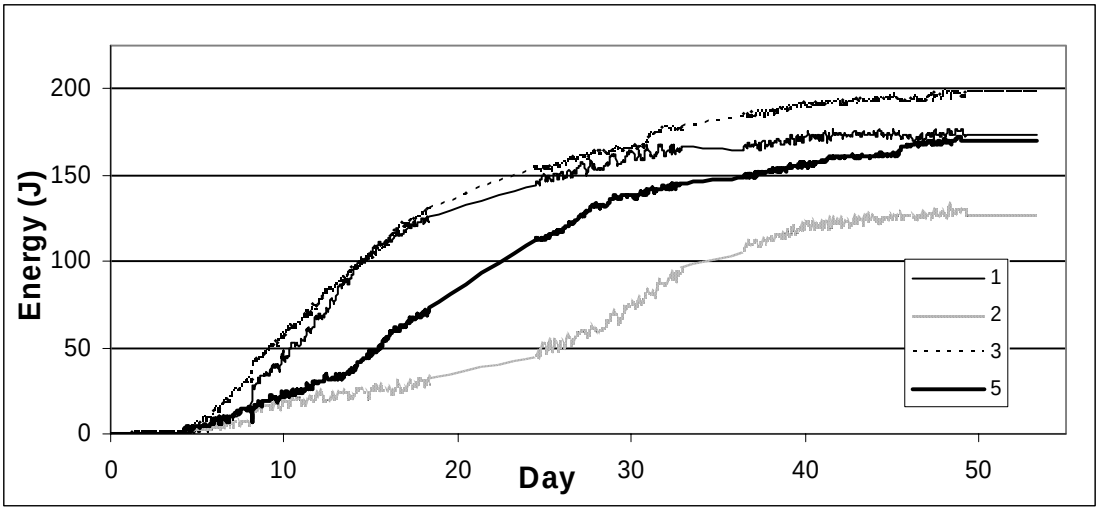


Figure 4-7. Total energy captured as electricity (assuming 35% conversion of biogas) from MFC followed by anaerobic digestion and then another MFC operation

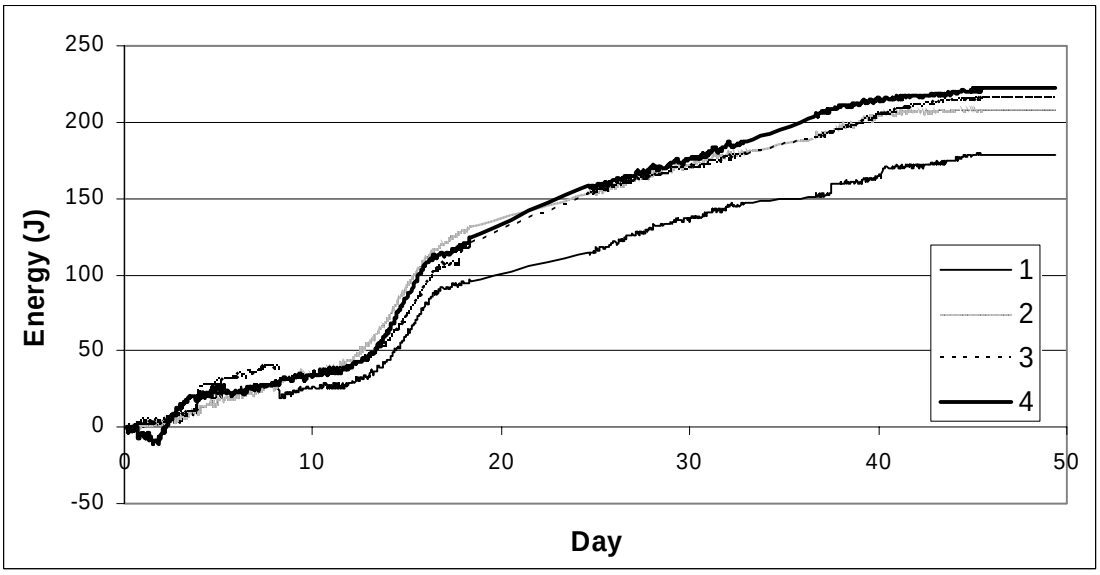


Figure 4-8. Energy captured as electricity from anaerobic digestion followed by MFC operation (assuming 35% conversion of biogas)

CHAPTER 5

SUMMARY AND CONCLUSIONS

These experiments show that microbial fuel cell operation is possible using dairy manure. The electrochemically active bacteria needed for MFC operation were naturally present in the dairy manure, readily colonized the MFC, produced power, and continued to operate without any non-manure additions or changes.

Removing the proton exchange membrane most likely caused the low MFC Coulombic efficiencies found in this study. However, it would lower the cost and internal resistance of the fuel cell, and while manure can be used as a 'free' substrate, additional COD destruction is not a problem and may be an advantage.

Power output and energy captured from the MFCs increased with increasing manure concentration (as measured by COD), suggesting MFC output was limited by the amount of readily biodegradable substrates present in the slurry more than the system design.

The mesophillic experiments showed a significant improvement in the MFC power output when operated at 37^{o}C versus 20^{o}C. However, it will need to be determined if this is only because the microorganisms degrade the substrates more quickly at higher temperatures, which generates higher currents and therefore power. If this is the case, the same amount of energy may be collected at any temperature, it would just take longer at the lower temperatures. Also critical to deciding if elevated temperatures should be explored will be to determine if the increase in the energy produced by the MFCs offsets the amount of energy that must be added to maintain mesophillic conditions. The MFCs did not operate effectively at 55^{o}C, implying that

thermophillic operation possibly inhibits and kills the electrochemically active bacteria.

The combined studies of MFCs with anaerobic digestion demonstrate that MFCs can operate before AD with little effect on biogas yield, and that MFCs can still operate after AD, but at a reduced rate. Also, when the manure was in a MFC before AD, in all cases it leveled out the rate of biogas production and in some of the trials caused biogas production to begin sooner, indicating the possibility of active microbial communities in MFCs that can jump start biogas production in AD.

For a final comparison of anaerobic digestion and microbial fuel cells, the volumetric power density of AD is approximately 400 W/m^3. The highest performing MFC has achieved 250 W/m^3 (stacked design, Rabaey et al. 2003), while the current MFC average is 40 W/m^3 (Pham *et al.* 2006). The same MFC design using swine manure was 6.5 W/m^3 (Min *et al.* 2005), and this study had a maximum of 4.5 W/m^3. At these seemingly small power outputs, one may wonder why MFCs may be a compliment to anaerobic digestion. However, MFCs have several advantages that eventually may make them an attractive addition/alternative to AD. For example, MFCs: 1) directly produce electricity eliminating the need for gas cleanup or costly engine-generator sets, 2) they can operate on dilute waste streams, and 3) do not need heat addition to maintain temperature.

When starting to look to the farm scale for MFC implementation, there is good potential to take advantage of the biodegradeable substrates in manure storage facilities, both those without AD and those that store digested manure. Anaerobic digestion is adversely affected by temperature reduction below about 30^oC. However, the MFCs operated effectively at temperatures below this (20^oC). This will allow MFCs to operate without the increased energy demand of elevated temperatures, making it suitable for use in ambient temperature manure storage systems. In

addition, most AD on farms only operate with a retention time of 20 days, at much higher COD concentrations, leaving more COD in the output stream, which can then be used by a MFC to generate electricity. The remaining COD and long residence time of these storage ponds may be an ideal place for MFC operation. Determination of the optimal design for this system is a remaining engineering opportunity. Whether the fuel cell is floating, in a pipeline, or a variation on a sediment MFC, has yet to be determined.

Microbial fuel cells still have much research and development needed to build optimal designs at a reasonable cost, but present another possibility to take advantage of a "waste" product and become part of a suite of renewable energy solutions that will displace fossil fuel based electricity while concurrently decreasing some of the environmental impacts of waste streams. "The future of the livestock-environment interface will be shaped by how we resolve the balance of two demands: for animal food products on one side and for environmental services on the other" (FAO 2006).

CHAPTER 6

FUTURE WORK AND RECOMMENDATIONS

Much work remains to be done before microbial fuel cells will be ready for industrial scale-up. If this system were scaled up to provide 1KW of power, it would require 8600 square meters of anode surface area (116 mW/m^2). Even the highest output MFC system to date (3600 mW/m^2) would still need 280 m^2. Using a simple approximation of flat panels 2 cm apart with microbes on both sides (98 m^2/m^3), our design would require 88 m^3, while the high performance MFC would need 3 m^3.

Therefore, an important area of research is to optimize configurations that will provide more power per anode surface area. As mentioned in the literature review, some improved configurations are already under development, such as stacked MFCs that increase overall voltage or current (Aelterman et al. 2006), or using porous anodes and reduced electrode spacing (Cheng et al, 2006), among others. These and other designs have done much to improve power output and efficiencies, but more work remains to overcome the obstacles of continuous operation and increased power output. A specific challenge with livestock manure operation is the high solids content, which may lead to clogging. Possible alternatives that could be examined would be determining the optimum porosity of anodes or operating MFCs only on separated manure liquids.

As an extension to the experiments conducted in this research, it would be valuable to design a MFC that could operate at the same time as AD to determine it the MFC could take advantage of the increased operating temperature and active microbial communities present during AD, which may provide more substrates for the electrochemical bacteria. However, this may also have more effects on biogas

production. It may also be advantageous to examine a continuous system that more approximately represents an operating AD system. This could be done with in-line continuous MFCs before, during, or after the AD process. Also, as MFCs become better optimized to recover more energy, the effects on biogas production during AD may become more significant, because more of the substrate is being converted into electricity leaving less for methanogen conversion.

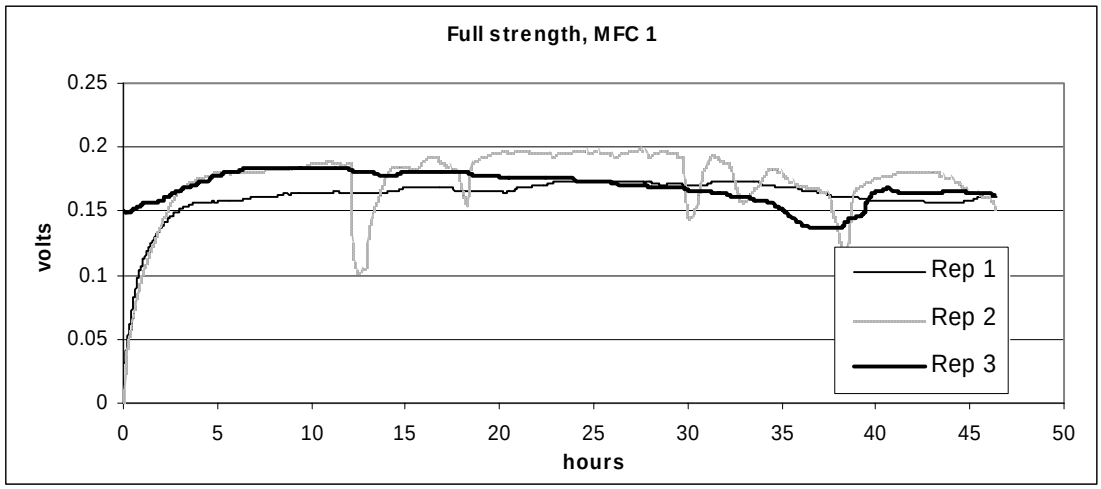
Another vital area of research is developing improved and less expensive cathodes. Here again, work is already underway through projects such as Cheng et al. (2006), where reduced precious metals and even non-precious metals are used with little reduction in performance. Further research should also consider further the removal of the proton exchange membrane. Without the PEM, there is a reduction in internal resistance leading to a five fold increase in power outputs (Liu and Logan 2004). Concurrently, there is more aerobic destruction of the substrate, leading to lower coulombic efficiency. However, if COD destruction is important and if there is an abundance of 'free' substrate, such as wastewaters or manures, then the added expense of a PEM may not be necessary. It needs to be determined how quickly the cathode catalyst becomes degraded and needs to be replaced without a proton exchange membrane, which will vary depending on the substrate in use. These factors should also be analyzed economically to optimize the MFC expense versus power production.

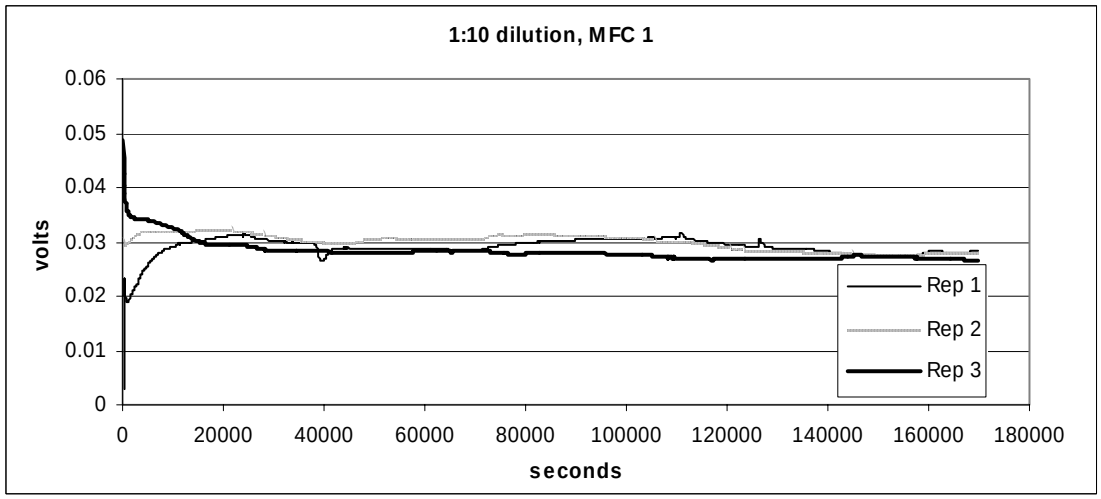
Another key step in developing microbial fuel cells will be improving the understanding of the microbial ecology and electrical generation process in order to optimize operating conditions and microbial communities. Tradeoffs between operating temperature, pH, preferred substrates, and other operational conditions can then be further examined to optimize energy and other external inputs with the value of the electrical energy produced. Understanding the ecology can also lead to

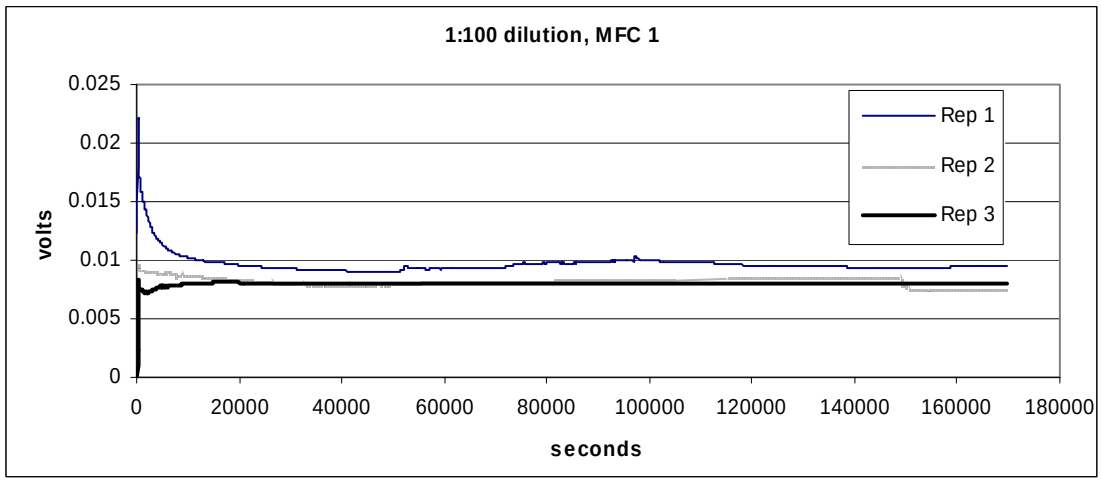
developing bacterial mixtures that optimize electrical generation and can quickly colonize MFCs to reduce startup time. Also, further examination of methanogenic bacteria that appear to be utilizing nanowires (Logan and Regan 2006) may reveal that methane production and biological electricity generation may be concurrently possible.

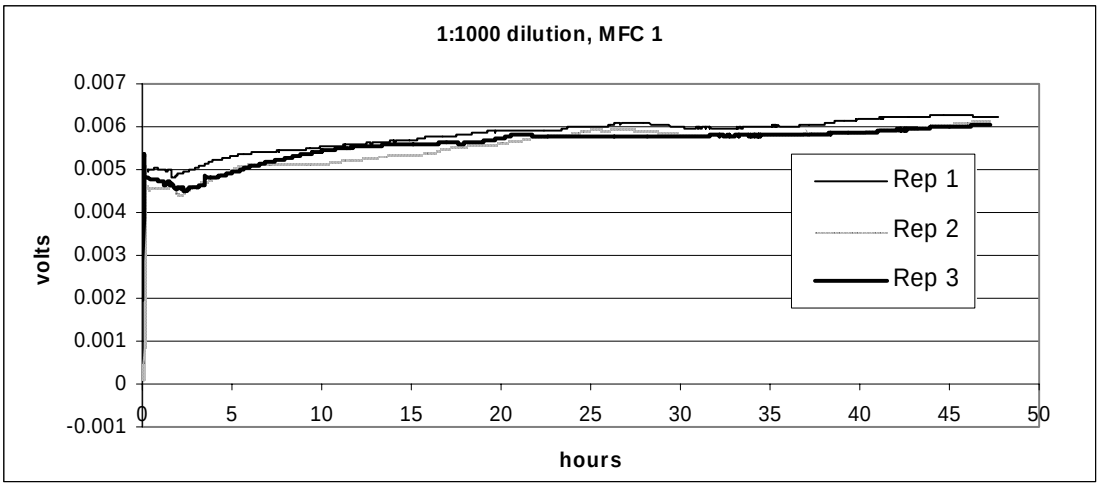
APPENDIX

COD Variation Experiment Voltage response curves, MFC 1

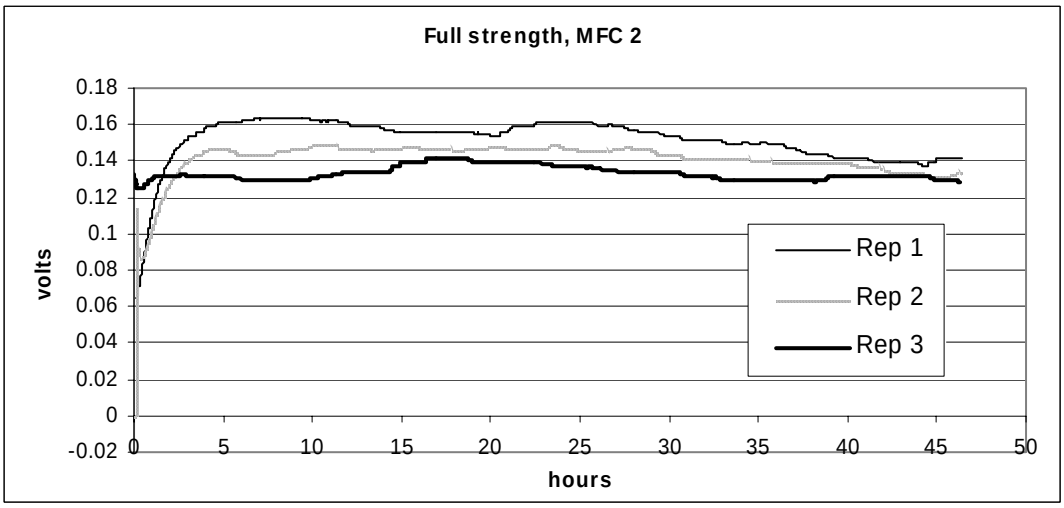


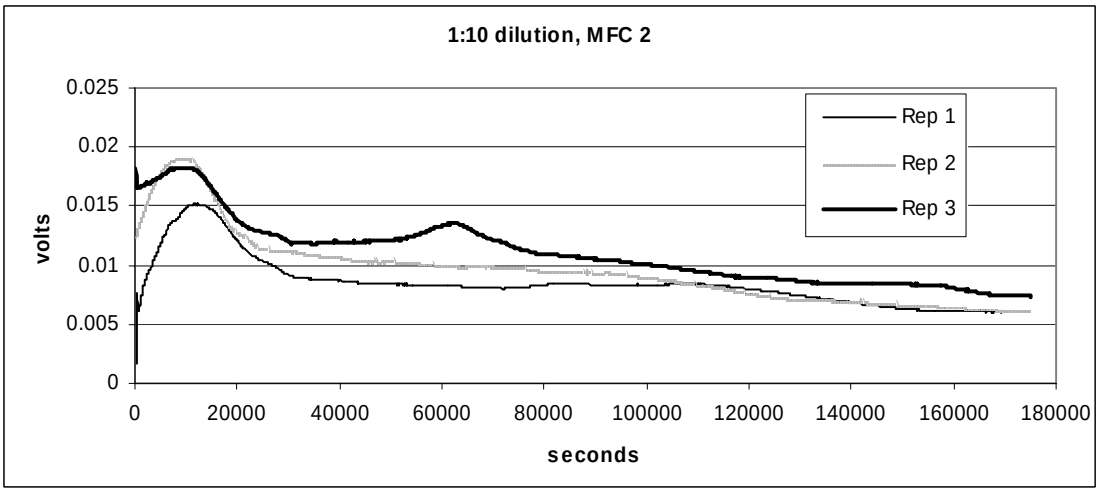


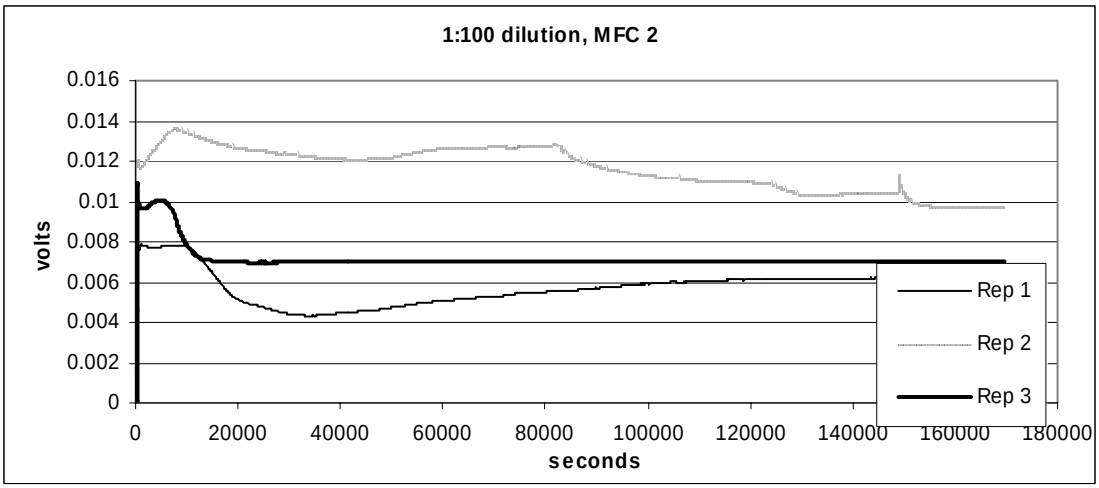


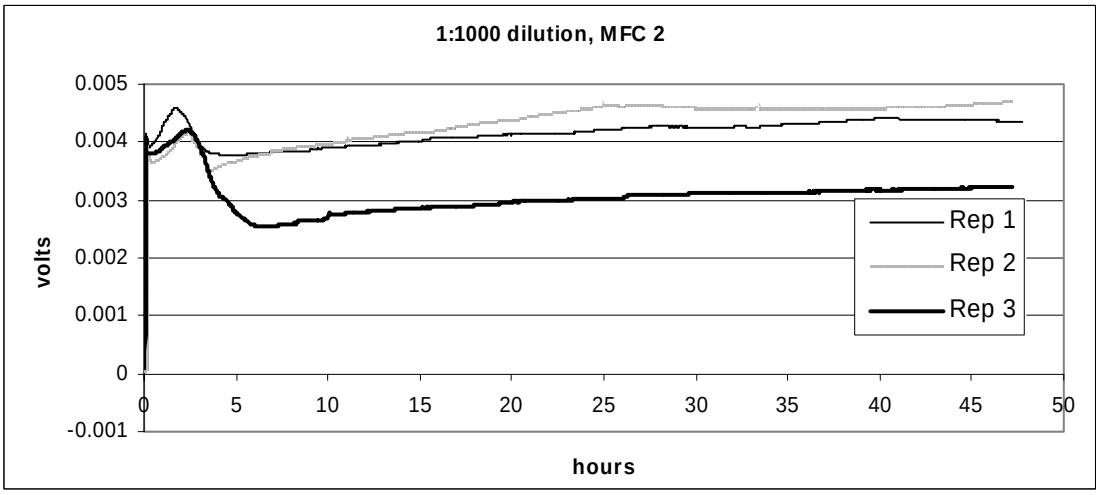


COD Variation Experiment Voltage response curves, MFC 2

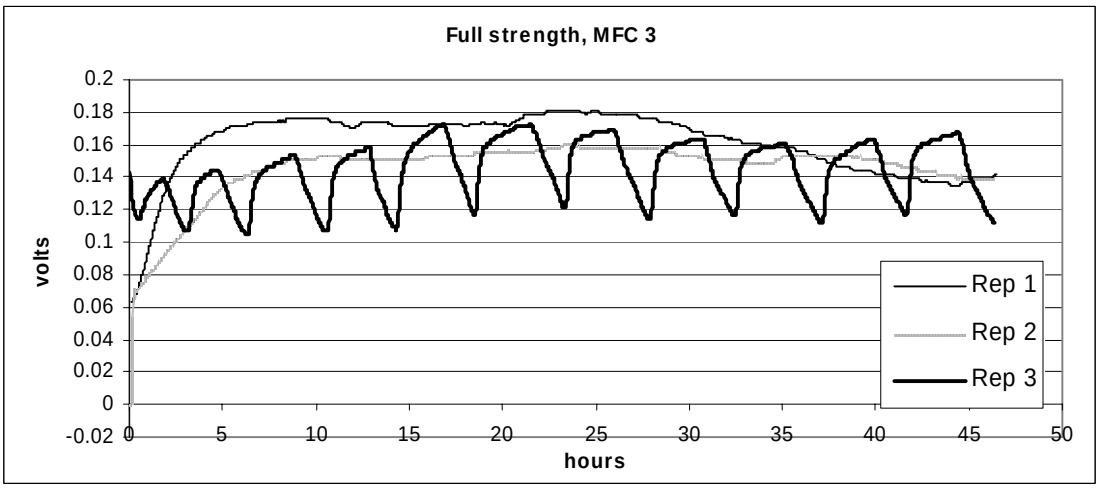


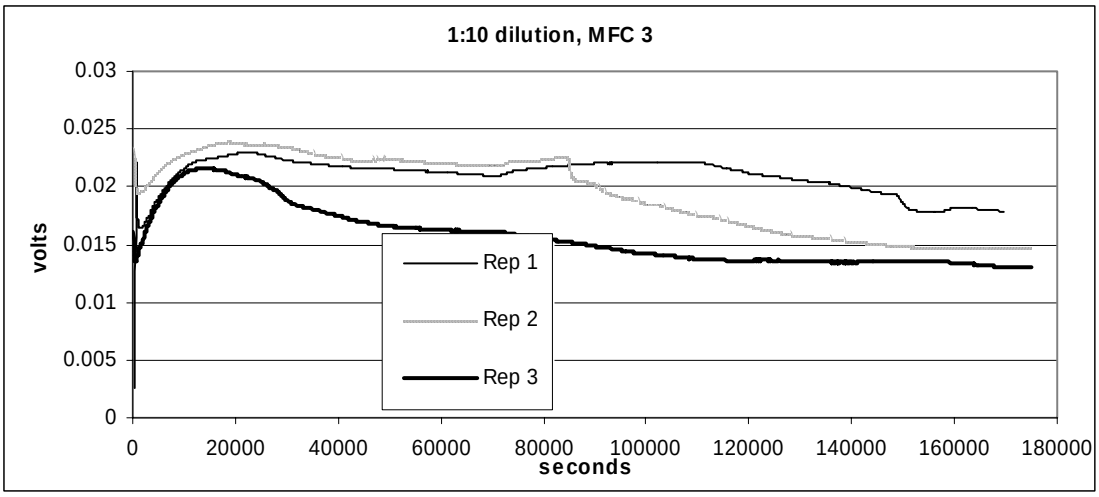






COD Variation Experiment Voltage response curves, MFC 3




1:10 dilution, MFC 3
volts
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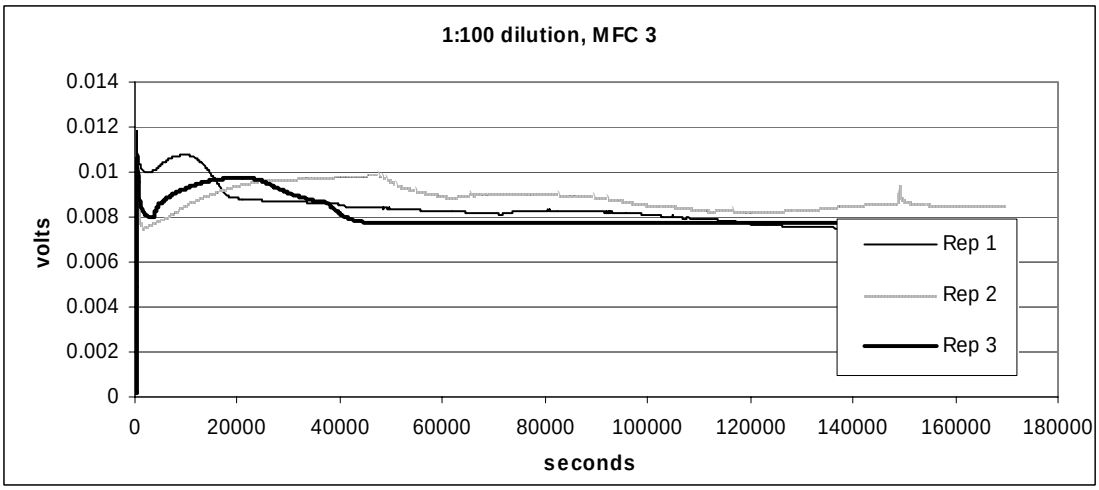
0.025
0.02
0.015
0.01
0.005
0
0
20000
40000
60000
80000
100000
120000
140000
160000
180000
seconds
Rep 1
Rep 2
Rep 3


1:100 dilution, MFC 3
volts
0.014
0.012
0.01
0.008
0.006
0.004
0.002
0
0
20000
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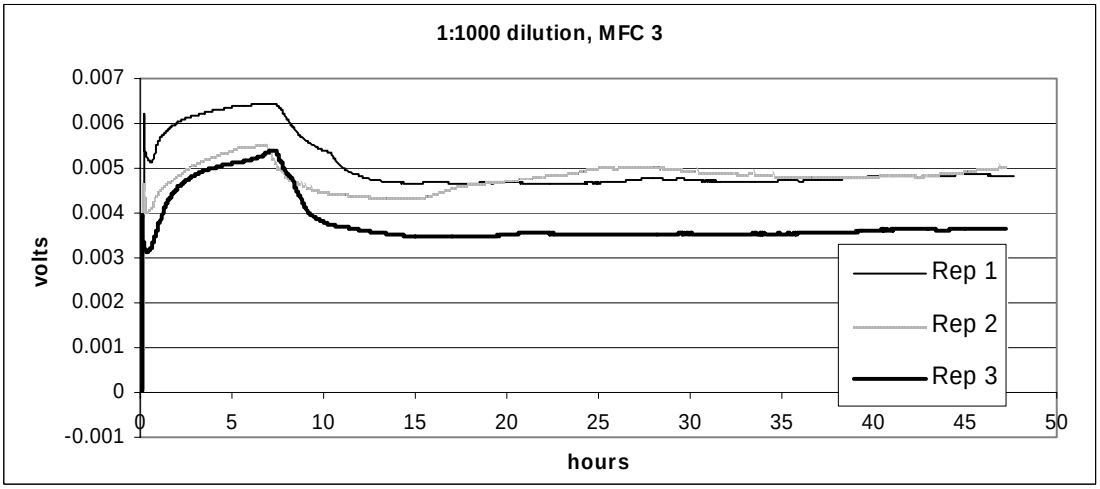
80000
100000
120000
140000
160000
180000
seconds
Rep 1
Rep 2
Rep 3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volts
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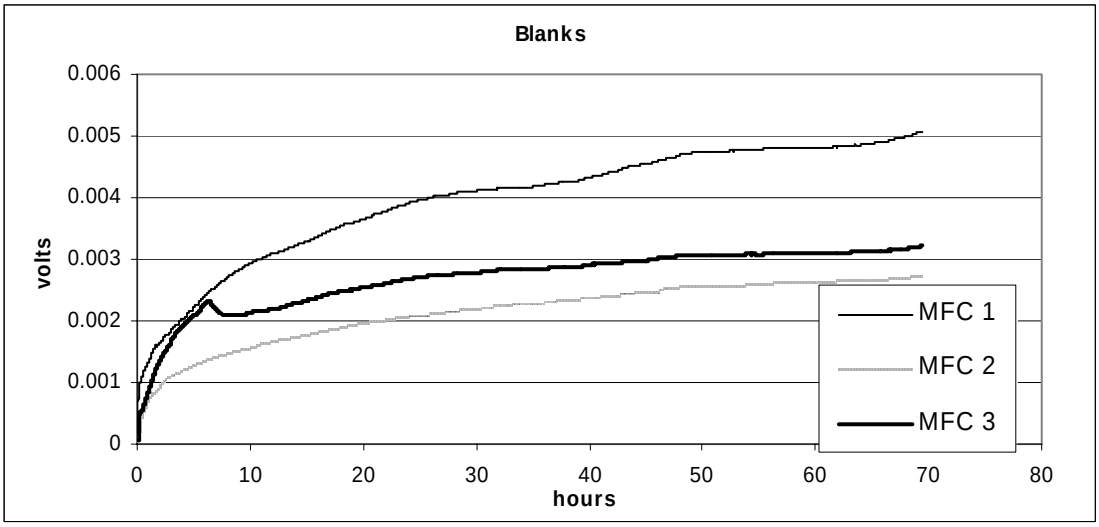
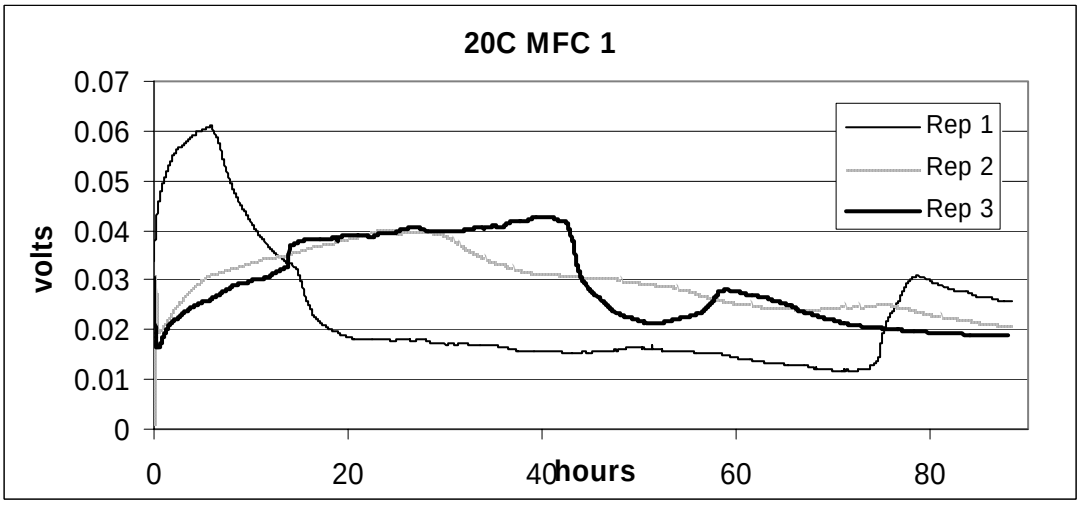
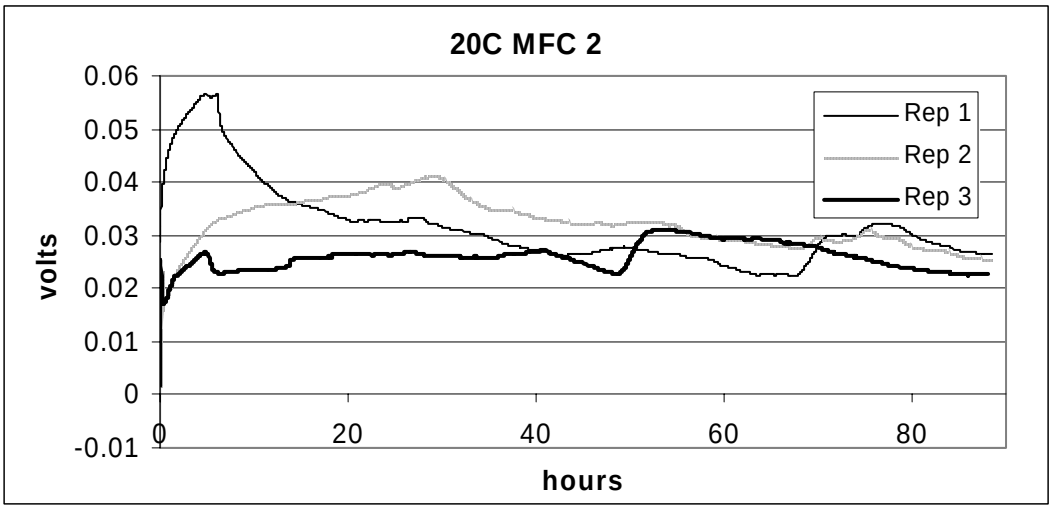
0.005
0.004
0.003
0.002
0.001
0
-0.001
0
5
10
15
20
25
30
35
40
45
50
hours
Rep 1
Rep 2
Rep 3

COD Variation Experiment Voltage response curves, blanks



Temperature Variation Experiment Voltage Response curves, 20^{o}C

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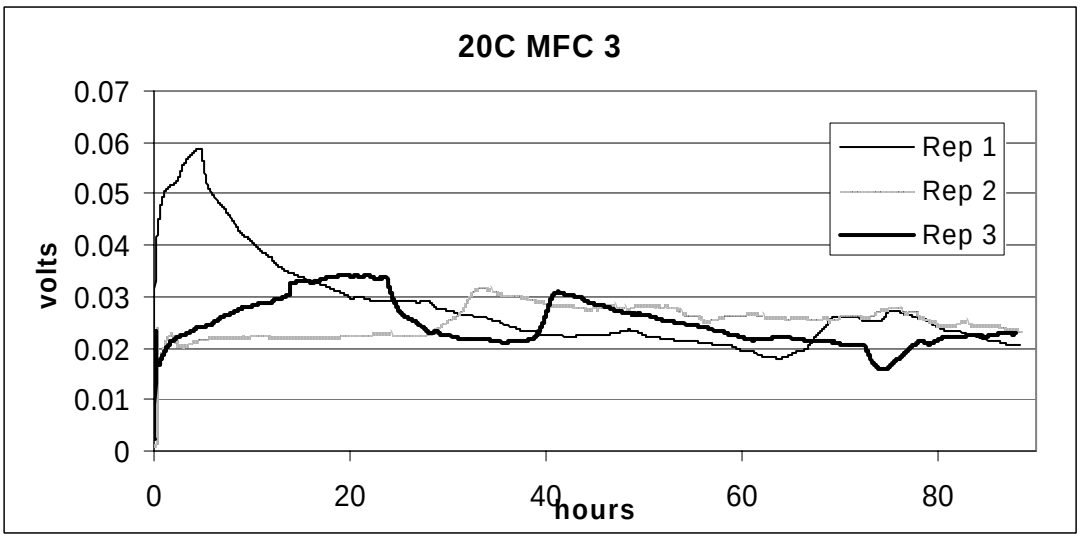
0.03
0.02
0.01
0
-0.01
volts
0
20
40
60
80
hours
Rep 1
Rep 2
Rep 3


20C MFC 3
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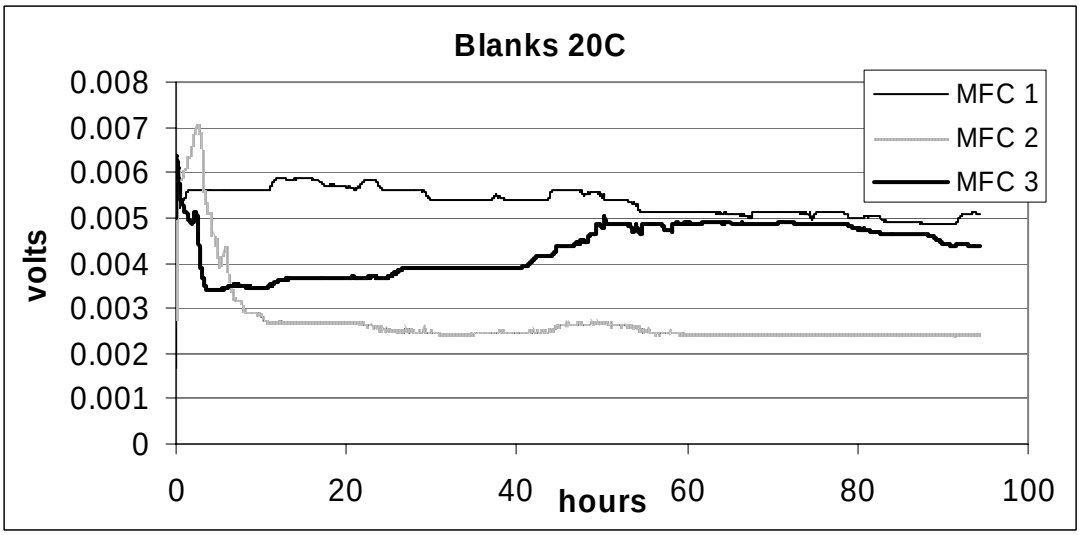
volts
0
20
40
60
80
hours
Rep 1
Rep 2
Rep 3


Blanks 20C
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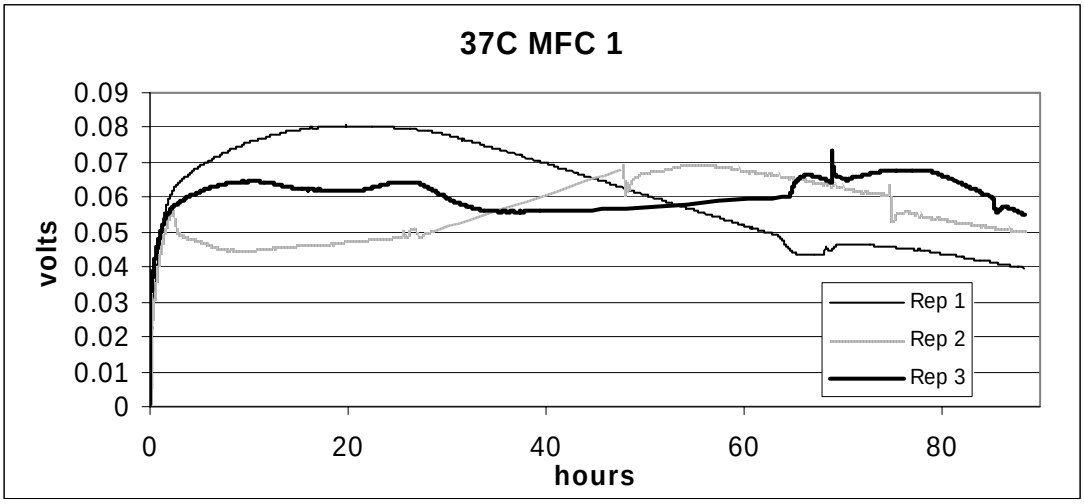
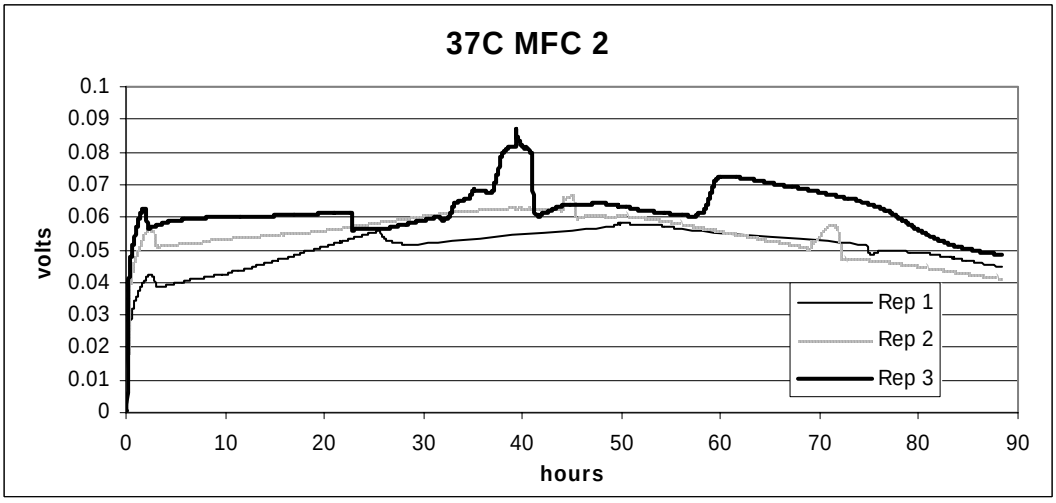
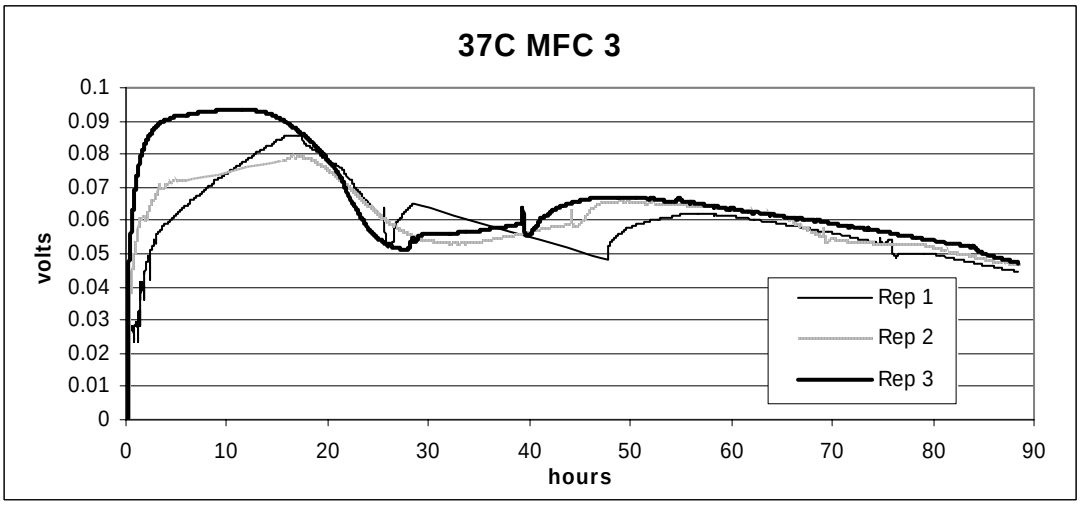
0.006
0.005
0.004
0.003
0.002
0.001
0
volts
0
20
40
60
80
100
hours
MFC 1
MFC 2
MFC 3

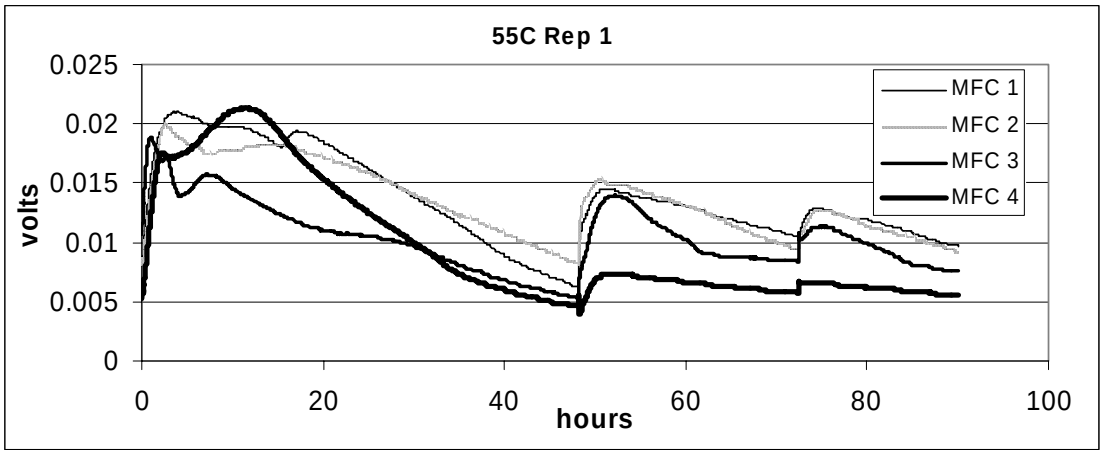
Temperature Variation Experiment Voltage Response curves, 37^{o}C

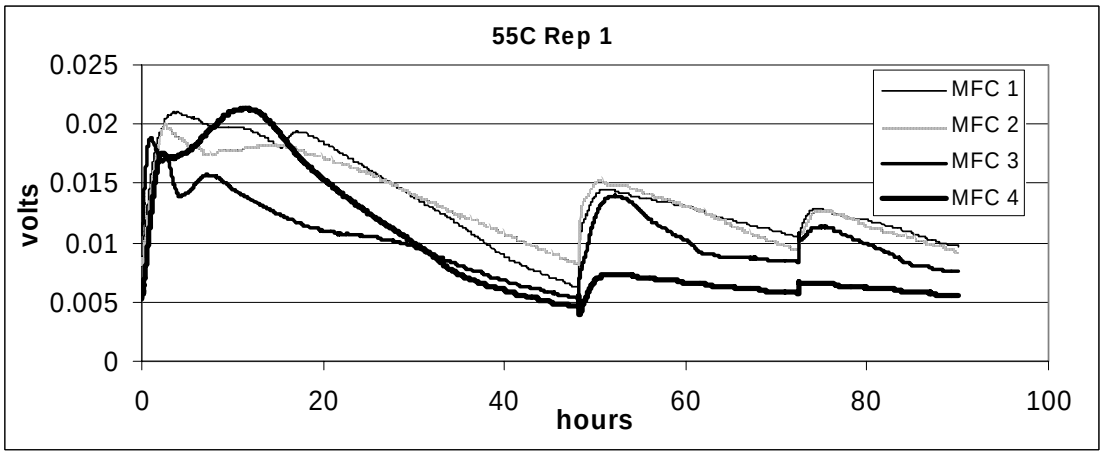


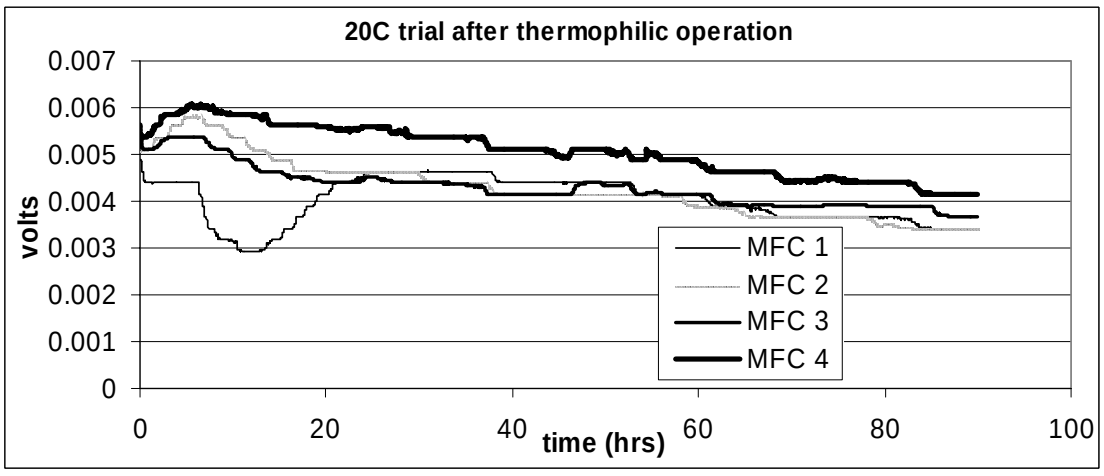




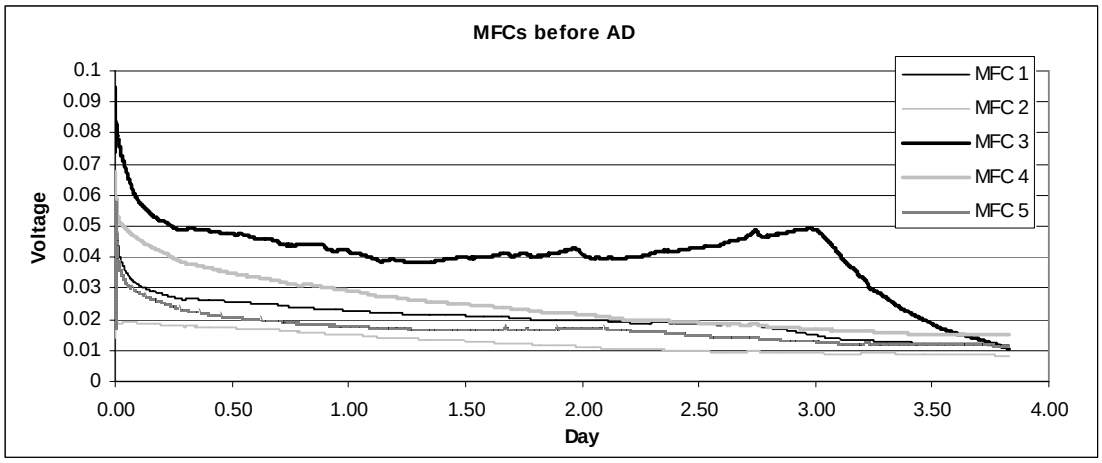
Temperature Variation Experiment Voltage Response curves, 55^{o}C

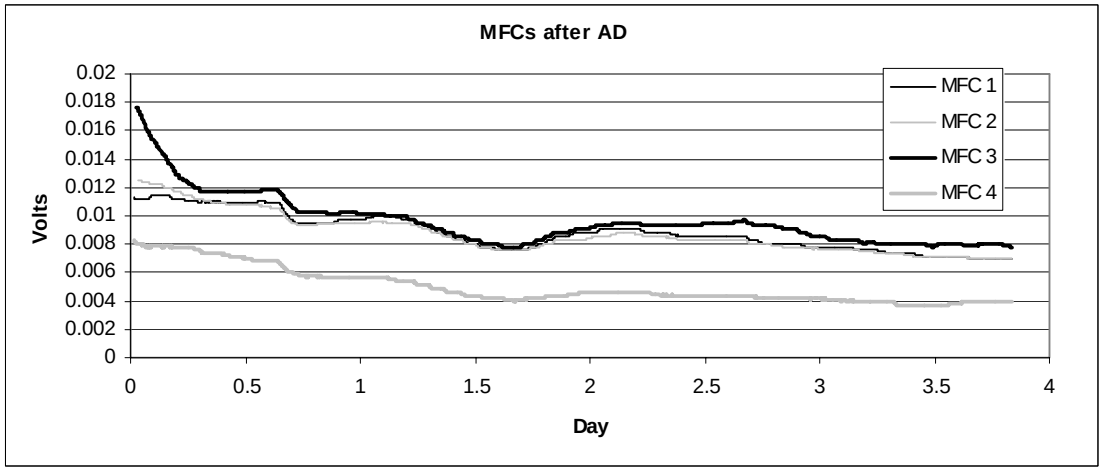


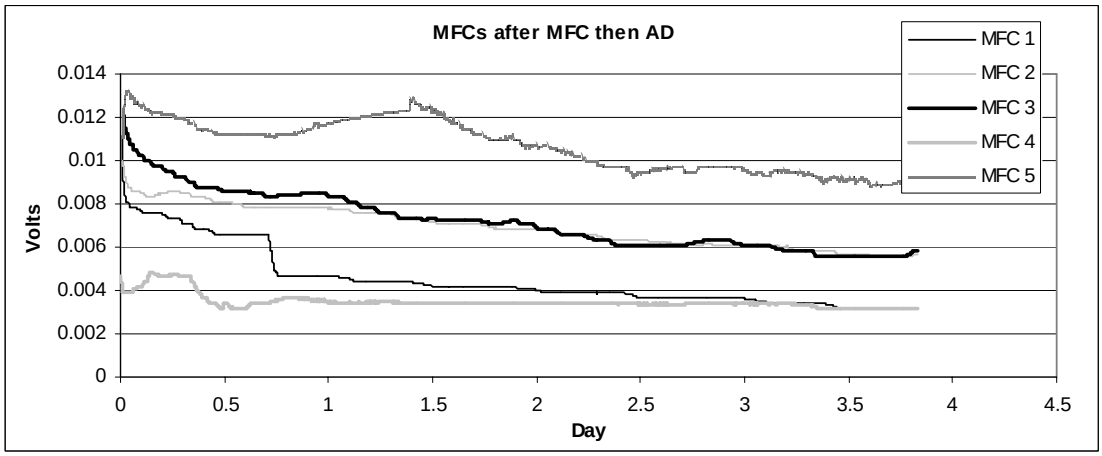




MFC operation results from MFC/AD experiment, 20^{o}C







COD-based biogas production estimation

	Equation			
Initial pressure - atmospheric (P1)		101325	Pa	
Target pressure - delta P (pressure to increase or gauge pressure)		50663	Pa	
Bottle volume		120	mL	
Sample volume		20	mL	
Headspace volume (V1)	Bottle - Sample volume	100	mL	
Final pressure - with biogas (P2)	Initial + Target pressure	151988	Pa	
Final volume of gas if it was compressed (V2)	P1*V1/P2	66.7	mL	
Volume of gas increased in the headspace - delta V	V1-V2	33.3	mL	This volume produces the final pressure, so this is the volume of biogas produced.
Target biogas yield		33.3	mL	
Methane %		60%	%	Assumed
Target methane yield	Target biogas yield * Methane %	20.0	mL	
mL CH4/g COD @ 35^{o}C		395	mL/g	Assumed
COD mass destroyed	Target methane yield / (mL CH4/g COD)	0.051	g	
Biodegradable COD		50%	%	Assumed
Total COD mass needed in substrate	COD mass destroyed / Biodegradable COD	0.10	g	
COD concentration in substrate		80	g/L	from sample characterization file
Substrate volume needed	Total COD / COD concentration *1000	1.27	mL	Assume density of manure equals water
Water needed	Sample - Substrate volume	18.73	mL	

Biogas composition determination

Equations

Initial moles $N_2 = P_i*V*XN_{2i}/(R*T_i)$
Initial moles $CO_2 = P_i*V*XCO_{2i}/(R*T_i)$
Moles $CH_4 = P_f*V*XCH_4/(R*T_f)$
Moles $CO_2 = P_f*V*XCO_2/(R*T_f)$
Moles $N_2 = P_f*V*XN_2/(R*T_f)$
% $CH_4 = nCH_4/(nCH_4+nCO_2-nCO_{2i})$

Molar fractions (Xn) obtained with the GC.
Final total pressure (P_f) is the raw pressure obtained with the transducers (not corrected for atmospheric pressure or temperature changes).

Trial	Final total pressure (atm)	Initial moles N_2	Initial moles CO_2	Fraction CH_4	Fraction CO_2	Fraction N_2	Moles CH_4	Moles CO_2	Moles N_2	% CH_4 sample	Average & St. Dev.
AD 1	1.5640	0.0029	0.0009	0.1290	0.2304	0.6472	0.0008	0.0014	0.0040	62.25%	
AD 2	1.6320	0.0029	0.0009	0.1208	0.1930	0.5163	0.0008	0.0012	0.0033	71.91%	64.49%
AD 3	1.6491	0.0029	0.0009	0.1330	0.2215	0.5934	0.0009	0.0014	0.0038	63.27%	0.0507
AD 4	1.6640	0.0029	0.0009	0.1426	0.2359	0.6132	0.0009	0.0015	0.0040	60.55%	
Refrig 1	1.6600	0.0029	0.0009	0.1285	0.2316	0.5332	0.0008	0.0015	0.0035	59.28%	58.31%
Refrig 2	1.6468	0.0029	0.0009	0.1530	0.2526	0.5823	0.0010	0.0016	0.0038	58.59%	0.0114
Refrig 3	1.5885	0.0029	0.0009	0.1165	0.2375	0.5627	0.0007	0.0015	0.0035	57.06%	
After 1	1.5199	0.0029	0.0009	0.1061	0.2446	0.5840	0.0006	0.0015	0.0035	54.63%	
After 2	1.4292	0.0029	0.0009	0.1060	0.2444	0.5701	0.0006	0.0014	0.0032	57.64%	56.76%
After 3	1.5710	0.0029	0.0009	0.1189	0.2375	0.5627	0.0007	0.0015	0.0035	58.04%	0.0152
After 5	1.5135	0.0029	0.0009	0.1111	0.2419	0.5899	0.0007	0.0014	0.0035	56.74%	
Uncon 1	1.4920	0.0029	0.0009	0.1101	0.2447	0.5786	0.0006	0.0014	0.0034	56.36%	
Uncon 3	1.5270	0.0029	0.0009	0.1153	0.2429	0.5574	0.0007	0.0015	0.0033	56.99%	56.09%
Uncon 4	1.4893	0.0029	0.0009	0.1136	0.2495	0.5820	0.0007	0.0015	0.0034	55.89%	0.0080
Uncon 5	1.5642	0.0029	0.0009	0.1221	0.2516	0.5645	0.0008	0.0015	0.0035	55.10%	

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