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THE EFFECTS OF CARRYOVER ON ELECTRICITY PRODUCTION AND CELLULOSE DEGRADATION IN MICROBIAL FUEL CELLS

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ABSTRACT

Due to environmental concerns associated with fossil fuel mitigation and political volatility of oil-producing countries, and more recently with the advent of rising fuel costs, biomass energy sources have become of great interest. One of the newest ways in which to harness the energy in biomass is through the use of a microbial fuel cell (MFC). MFCs are a new technology with promising application in the field of wastewater treatment amongst many others. MFC research has focused primarily on the treatment of easily degradable soluble carbon sources, but biomass is known for its recalcitrance, and MFC treatment of particulate substrates should be explored more in depth for the best method of treatment.

In previous testing of cellulose conversion to electricity in an MFC, hydrolytic and fermentative bacteria have been found to predominately reside near the suspended cellulose that they are degrading, and not on the anode of the MFC. Therefore, one of the methods to keep those organisms within a batch-fed system would be to carry over some of the substrate from the previous cycle into the next. In this study, air-cathode single-chamber bottle MFCs were used to evaluate the ability of both sludge and binary (*Geobacter sulfurreducens* and *Clostridium cellulolyticum*) inocula to degrade cellulose, remove COD, and generate power, comparing for each inoculum a system that had carryover from the previous cycle and a system with no carryover.

The sludge-inoculated MFCs proved to be superior in the generation of maximum power (41.4 mW/m^2 and 39.0 mW/m^2 without and with carryover, respectively) to the

binary-inoculated MFCs (19.4 mW/m^2 and 13.7 mW/m^2 without and with carryover, respectively). Higher transient values were seen in the earlier cycles (62 mW/m^2 and 44 mW/m^2 for sludge-inoculated MFCs without and with carryover, respectively and 28 mW/m^2 and 19 mW/m^2 for binary MFCs without and with carryover, respectively), but the performance generally appeared to be more stable at the later cycles. Gas composition for the sludge MFCs was monitored and methane was discovered in the headspace of only the system with carryover. For both inocula, the MFCs with carryover demonstrated the highest removal of cellulose (95% for the sludge-inoculated MFC and 90% for the binary MFC). Although carryover did promote higher fermentation rates, this operational strategy also created a system with acidity issues, needing the pH adjusted almost every other day to obtain pH > 6. The highest coulombic efficiency (CE) was observed in the binary MFC with no carryover (11.4%). The rest of the MFCs had CEs of ~ 3%. Acetate was found in abundance in the binary MFC with carryover both before (1.7 mM) and after (10.1 mM) the analyzed run, but not in any other MFCs. This residual acetate concentration suggests that the *G. sulfurreducens* population was not able to keep up with the fermenters. Real-time PCR with samples from the binary reactors showed that carryover was effective at retaining many more fermentative organisms than the MFC without carryover, showing an accumulation over time of these fermenters in the binary MFC with carryover. Although there appeared to be a trade-off between power density and cellulose conversion in the binary reactors, this trend was less evident in the sludge-inoculated reactors.

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1

Introduction

The prevalence of cellulose in many waste streams and as a main component in energy crops, coupled with its recalcitrance to biological conversion, makes it a necessary and desirable substrate to evaluate and optimize within biological reactors. The economical and environmental impetus of using waste streams and energy crops for energy production warrants the attention and focus of the research community to solve the problems set forth by fossil fuel prices generated due to high demand, environmental concerns about upsetting the earth's carbon balance more so than already done, and the implicit nonsustainability of fossil fuel reliance.

One strategy for energy recovery from cellulosic wastes and biomass involves conversion to electricity in a microbial fuel cell (MFC). Many soluble substrates and waste streams have been successfully treated with MFCs, measured in terms of removal of chemical oxygen demand (COD), power generation, and overall system efficiency. There has been considerably less research on cellulose-fed systems, though interest in this is beginning to emerge. Cellulose conversion in an MFC introduces a complication in the need for hydrolysis of an insoluble electron donor in suspension and reduction of an insoluble electron acceptor at the anode. This creates an interesting ecological constraint that must be addressed in reactor operation to allow effective conversion.

The hypothesis of this research is that the operation of a cellulose-fed MFC with the deliberate retention of medium between feedings will increase the cellulose-degrading

ability of the system. This operational strategy should address the ecological dilemma by retaining a high density of suspended cellulose-degrading microbes. Also, this retention may influence power production by creating less of a lag time before power production due to easily degradable fatty acids retained in the carryover from one cycle to the next, and a higher flux of fermentation-derived fatty acids for use by exoelectrogenic anode-reducing microbes.

Chapter 2 presents the background for this study. The impetus of environmental and economical concerns and the place of cellulose in energy production are addressed, leading into the background of MFC technology and previous research efforts related to this study. Chapter 3 details the experimental methods used in this study. Chapter 4 presents the results for each of the tests done to evaluate the MFCs' performance. Chapter 5 discusses these results in detail and presents ideas for future research endeavors. Finally, Chapter 6 lists the conclusions of this study.

Literature Review

2.1 Alternative Energy

2.1.1 Impetus for Study

Throughout the world there is intense interest in evaluating and implementing alternative energy sources. This work is driven by both environmental and economic concerns. Through public and even political impetus, reports identifying the concerns of global warming and increasing atmospheric carbon levels have been increasingly prevalent. The levels of carbon dioxide in the atmosphere have risen appreciably since the use of fossil fuels (Meehl 2007). Although some atmospheric modeling results show these increases as not harmful to the environment (Robinson 2007), many researchers feel that this increase is harmful and could only be due to anthropogenic carbon emissions from the burning of fossil fuels (Hegerl 2007). This increase in carbon dioxide has been implicated as a cause of global warming due to the greenhouse effect, which is further exacerbated by other products of fossil fuel combustion such as nitrous oxides and incompletely combusted hydrocarbons (Houghton 2005). Global warming is of concern for many reasons, for example the increase in sea level due to the melting of the ice caps. This particular problem that would cause flooding in many coastal areas and only be

exacerbated by the reduction in the size of the ice caps which would reflect less solar energy into to space. This increase in solar energy would then increase global temperatures, melting more of the polar ice, which would then increase sea level even further (Tjoelker and Zhou 2007). One of the remediation tactics for the problem of global warming is to reduce the release of carbon emissions by using carbon-neutral and possibly carbon-negative fuel sources.

The environmental imperative to switch to renewable energy sources is straightforward, but economically the benefits have been less apparent. The rising costs of fossil fuels paired with the economic and political volatility of the sources of much of the petroleum have made renewable energy an even more important choice for the United States to employ. Besides price volatility, the demand of fossil fuels along with scarcity of petroleum has led to oil prices reaching over $100 a barrel, and although some projections have these prices decreasing within the next year (U. S. Department of Energy 2008), the rise has many consumers feeling the pains of using a fuel source from a mostly foreign market. The projected energy demands of the world have been estimated to grow 57 percent between 2004 and 2030, further driving up energy prices due to this increased demand (U.S. Department of Energy 2007). Having a biomass-based fuel source should serve to negate some of these demand-driven economics by lessening the United States' presence in the petroleum world marketplace. Besides obtaining a reliable fuel source, feedstocks for bioenergy production have the ability to provide a new labor industry in rural areas where economic recession is most readily felt (Perlack RD 2005).

2.1.2 Carbon neutrality

A carbon-neutral fuel is defined as a fuel source that has no net carbon output associated with its use; a carbon-negative fuel actually removes carbon from the atmosphere by its use, usually through some sort of carbon fixation (Mathews 2008). Biomass is an example of a carbon-neutral fuel. Its utilization, whether through combustion, gasification, or microbial conversion to an intermediate energy carrier or directly to power in an MFC, releases CO_2. However, the production of biomass involves the assimilation of CO_2 through autotrophic carbon fixation, resulting in no net release of CO_2 (Faaij 2006).

Biomass is the second largest source of renewable power in the United States and it is slated to surpass hydroelectric power as the number one source of renewable energy in the next decade (Perlack RD 2005). Although concerns have been raised about the production of biomass as an energy crop (and not for food) (Solomon 2007), an in depth study done at the Oak Ridge National Laboratory explored this issue and estimated that 1.4 billion tons of biomass could be sustainably produced in the United States, displacing over thirty percent of the current petroleum utilization (Perlack RD 2005). The report accounted for many waste residues, but did not consider municipal wastewater as a source of energy. There is a considerable amount of energy in wastewater and its treatment is an optimal application for MFCs to produce electricity given that wastewater is free and conventional means of treatment have a sizeable energy deficit (Logan and Regan 2006).

2.1.3 The Role of Cellulose

A main component of biomass is cellulose, which can be a significant fraction of many conventional waste streams such as paper industry waste, agricultural wastes (Demirbas 2003), domestic wastewater, municipal solid waste, and other industrial wastewaters (Mandels et al. 2004). Cellulose is a polymer of glucose molecules attached by β-1,4 linkages into long chains. These bonds, along with the bonds that are formed between and within these polymers, allow the formation of microfibrils that impact the recalcitrance of cellulose. Cellulose also does not naturally occur alone, but in the company of both lignin and hemicellulose, the former of which is even more difficult to breakdown and remove from biomass. Cellulose must first be freed from the other polymers surrounding it, then hydrolyzed or broken into smaller molecules. The glucose monomers are then further broken down by fermentation into organic acids, alcohols, hydrogen, and carbon dioxide. This breakdown can be done by a host of microorganisms utilizing many different enzymes to complete the task (Béguin and Aubert 1994).

2.2 MFCs

2.2.1 History and Present Concerns

In an MFC, microorganisms oxidize a substrate and transfer those electrons to an anode electrode. The electrons then flow through an external circuit, creating a useable current, and then reduce an oxidant at the cathode. A critical component of this system is

the ability to achieve extracellular electron transfer. This capability was discovered about a century ago by Potter (1911), who looked at potential decreases with the placement of a platinum electrode into an *Escherichia coli* culture. In the 1960s, this work was expanded by decoupling the half reactions present in this biochemical reaction to produce an electrical current. Performed with *E. coli*, these reactions showed very little power production until the addition of a mediator, which served to shuttle electrons between the cell and the anode of this simple system (Davis 2007). More interest was garnered into the 1980s, and mediators became of great interest. However, due to toxicity concerns (especially with treating waste streams that would be released into potable water sources) and cost (since the mediators were regularly broken down throughout the cycle), using exogenous mediators in an MFC is not thought to be a sustainable technology (Du et al. 2007).

Presently, much of the work in MFCs focuses on mediatorless systems, fuel cells which do not have to use an added mediator to transfer electrons to the anode. This was first demonstrated in 1999 by Kim et al. Such bacteria are known as exoelectrogens (Logan 2008), and an example of such a microorganism would be *Geobacter sulfurreducens* (Bond and Lovely 2003). The mechanism by which the electrons are transferred is being explored presently, and could involve the production of mediators by the cells themselves (Rabaey et al. 2004), the use of outermembrane cytochromes (Myers and Myers 2002), or the use of conductive filaments (known as nanowires) to transfer the electrons directly from the cell to the anode (Reguera et al. 2005, Gorby et al. 2006). The coulombic efficiency, or the number of electrons captured by electrical current divided by the number of electrons that are extracted from the substrate, is one of the

defining measurements in the performance of a fuel cell (Logan 2008), and is further explained in 3.5.2.

2.2.1 Technology Overview

An MFC is based on the fact that different redox potentials of chemical reactions can generate power, as in conventional batteries. Electron donor substrate at the anode is oxidized (an example of glucose is shown in Eq. 2.1, written in conventional format as a reduction), and these electrons travel to the cathode generating an electrical current. At the cathode, an electron acceptor is then reduced (as shown for oxygen in Eq. 2.2). The standard electromotive forces (E^0) are given in terms of NHE (normal hydrogen electrode, $E^0(H_2) = 0$).

$$\text{Anode: } 6CO_2 + 24H^+ + 24e^- \rightarrow C_6H_{12}O_6 + 6H_2O \quad E^0 = -0.014 \text{ V} \qquad \textbf{2.1}$$

$$\text{Cathode: } O_2 + 4H^+ + 4e^- \rightarrow 2H_2O \quad E^0 = 1.229 \text{ V} \qquad \textbf{2.2}$$

The difference between the redox potentials in this system is the maximum theoretical potential of the cell. To calculate the overall potential, the reduction potentials must be adjusted for pH, reactant and product concentrations, and temperature. There are many electrochemical losses that may further reduce this maximum value, such as, but not limited to, the internal resistance of the MFC and the microorganism's ability to degrade the feedstock.

MFC architecture generally consists of three main components, the anode, the cathode, and a membrane that separates the anode and the cathode but allows the flow of

protons from the anode to the cathode. The anode is a conductive material, often carbon based (carbon fiber brushes, carbon paper, graphite rods, etc.), and is submersed in an anaerobic environment. The anode material has to be able to carry a current as well as allow microorganisms to colonize on it; this colonization is also very important for the production of electricity in the fuel cell (Logan 2006; Logan, Cheng et al. 2007; Aelterman, Freguia et al. 2008). The cathode is often a limiting aspect of an MFC. The most common and feasible electron acceptor for large-scale application is oxygen. Other compounds such as ferric cyanide and permanganate can be used, with higher coulombic efficiencies (electron recovery), but pose problems such as toxicity to the microbes and regeneration issues after their use. Using oxygen as the electron acceptor has several drawbacks, one being that the chemical reaction at the cathode is slow, so a catalyst (either chemical or biological) is needed for the reaction to proceed at useful rates. This can prove to be costly, as the most common material used in this manner is platinum, but alternative catalysts such as lead dioxide and cobalt tetramethylphenylporphyrin have been shown to work as well, with lead producing 2-4X the power densities and cobalt slightly lower power densities (Morris et al. 2007; Cheng et al. 2006) compared to platinum catalysts on the cathodes. Also the infiltration of oxygen into the anaerobic anode chamber reduces the coulombic efficiency of the system. Biocatalysts on the cathode offer another interesting challenge, requiring the correct microbial communities to maintain a significant potential difference between the electrodes, allowing the flux of electrons (Logan 2006; Logan and Regan 2006; Logan 2008; Rabaey and Keller 2008; Rismani-Yazdi, Carver et al. 2008). A membrane is not always used in an MFC because it can reduce system performance by limiting the rate of proton transfer to the cathode

and the cost of the membrane can be prohibitive (Logan 2008). Such a membrane is necessary in a two-chambered MFC, but not in a single-chamber MFC with an air cathode (Cheng, Liu et al. 2006). One advantage of a membrane is that it can reduce oxygen diffusion into the anode chamber, thereby reducing electron loss due to aerobic respiration and increasing coulombic efficiency.

Most of the work with MFCs has been done using soluble substrates, but real wastewaters generally contain particulate materials with varying degrees of solubility and degradability. Likewise, other waste streams have a substantial portion of biomass and therefore are recalcitrant to biological degradation. One treatment process for these wastes is anaerobic digestion. For MFCs to be a viable technology, many feel that the anaerobic digestion "benchmark" (of energy or products produced per mass of substrate utilized) must be met. MFCs are thought to be a more viable technology where the environmental constraints (e.g. dilute waste stream, low temperature) preclude the use of the digestion technology to produce energy (T. H. Pham 2006; Du, Li et al. 2007).

2.3 Previous Related Work

Many studies have looked at the ability of glucose, the monomeric subunit of cellulose, to be broken down within an MFC (Rabaey et al. 2003; Chaudhuri and Lovley 2003; Niessen 2005; Catal, Fan et al. 2008; Lee, Parameswaran et al. 2008; Mohan, Manoj Muthu Kumar et al. 2008; Venkata Mohan, Veer Raghavulu et al. 2008). One study compared the complete electron balance for two systems with the same inoculum, with one fed acetate (a non-fermentable substrate) and the other fed glucose (a

fermentable substrate) (Lee, Parameswaran et al. 2008). This study showed that methane losses were appreciable for the glucose-fed MFC, and that coulombic efficiencies were almost 2 times higher (49% for glucose versus 71% for acetate) for a non-fermentable carbon source. Jung and Regan (2007) also reported a high methane to CO_2 ratio in glucose-fed systems relative to acetate-fed and lactate-fed reactors, and the emergence of fermentative community members in the glucose-fed reactors.

Several other studies have evaluated soluble biomass-derived products for electricity production in MFCs. Niessen et. al. (2004) looked at the ability of an MFC to degrade starch and produce electricity. Like cellulose, starch is a polymer made up of glucose molecules, but is much easier to degrade because the monomers are linked by α-1,4 and α-1,6 bonds (versus only α-1,4 linkages in cellulose). This study used a mediator (resazurine) to enhance electron transport. Molasses and glucose were also used to compare the current densities of the two cellulose-degrading species used. A poised potential was used to evaluate the system, ultimately showing that this system appeared to generate more current than conventional cathode/anode systems, perhaps due to overcoming conventional MFC constraints such as internal resistance (Niessen, Schröder et al. 2004). The same group of cellulose-degrading bacteria can also produce hydrogen, and then the in situ oxidation of that fermentation product for electricity production was demonstrated using a Pt catalyst at the anode (Niessen 2005). This work was cited as the first to be done looking at cellulose degradation for electricity generation in MFCs. However, the system was a departure from the main features of an MFC in that bacteria did not catalyze anode reduction.

Another study looked at a the conversion of hydrolysate from steam-exploded corn stover in an MFC (Zuo, Maness et al. 2006). This study used air-cathode MFCs, which have lower coulombic efficiency due to oxygen diffusion into the anode, but a greater potential for actual application due to higher power generation. The maximum current density found in this system was around 370 mW/m^2. This power density was lower than those reported in other studies using soluble sugars to imitate products of cellulose and biomass breakdown (Catal, Fan et al. 2008). This study showed power densities from glucose of 2160 mW/m^2 in air-cathode MFCs, although they had about half the volume of the previously mentioned study for the anode chamber.

Ren et. al. (2007) demonstrated the ability of a binary system, the cellulose-degrading *C. cellulolyticum* and the exoelectrogens *Geobacter sulfurreducens*, to produce electricity from soluble and insoluble cellulose, since no single organism has been reported to break down cellulose and produce power within an MFC (Ren, Ward et al. 2007). Another group evaluated the ability of microorganisms from rice paddy soil to break down cellulose and produce electricity (Ishii, Shimoyama et al. 2008). This group mainly looked at the diversity of the microbial community, but also showed a positive correlation between the electricity production and a neutral pH. Ishii et al. (2008) used rezazurin in their medium, which has been shown to act as a mediator, notably with *Clostridium cellulolyticum*, a cellulose-degrading microorganism (Sund, McMasters et al. 2007). A control for the rice paddy soil showed that the same amount of power was generated when the potential electron shuttle was omitted. Another study looked at the electricity-generating potential of an MFC inoculated from the rumen contents of a cow, utilizing cellulose as the feed stock (Rismani-Yazdi, Christy et al. 2007). This study

showed that electricity generation was possible using such an inoculum. Both of these studies (the rice paddy soil and the rumen inocula) incorporated l-cysteine in their medium, which has been shown to be a suitable substrate for electricity production in an MFC with wastewater inoculum (Logan et al. 2005). Ishii et al. (2008) argued that the concentration of l-cysteine in the system (0.5 g/L) was comparatively negligible to the concentration of cellulose (6 g/L) in the system. The rumen group did not address this potential source of current, although their cellulose:cysteine ratio (7.5 g/L to 0.5 g Cysteine · HCl/L) was higher. In all of these experiments, ferric cyanide was used as the electron acceptor in a two-chamber MFC employing a proton exchange membrane between the two chambers. Using this reactor configuration, the authors obtained maximum power densities of 59 mW/cm^2 (Ren et. al. for binary culture of *G. sulfurreducens* and *C. cellulolyticum* utilizing MN301, a recalcitrant cellulose), 10 mW/m^2 (Ishii et. al.), and 55 mW/m^2 (Rismani-Yazdi et. al. using milled cellulose). Utilizing a two-chamber system with aqueous oxygen at the cathode, Ren et al. (2007) observed power densities of 34 mW/m^2. Another group looked at the degradation of cellulose in a two-chamber fuel cell with aqueous oxygen at the cathode found a maximum power density of 35 mW/m^2 (Rezaei et al. 2007).

Venkata Mohan et al. (2008) looked at the effects of carryover on power production in an MFC. This experiment was carried out in a single-chamber MFC that had glucose-amended artificial wastewater circulated through the cell. It showed immediate power production with the retention of carryover. Looking specifically at what type of bacteria may be more prevalent in the carryover, Ren et al. (2008) preformed molecular analysis on their binary-culture systems to investigate spatial

heterogeneity of the cellulolytic and exoelectrogenic bacteria between suspension and anode biofilm. This study found through both real-time PCR and FISH (Fluorescence in situ Hybridization) that the fermenters were more closely associated with the cellulose particles in suspension, and not very numerous on the anode itself. The anode was where the bulk of the exoelectrogens were found (Ren 2008).

Based on all of this information, it was decided to look at an experiment that would tie carryover (a retention of cellulose-degrading organisms) with cellulose degradation, to see if carryover would positively affect the cellulose degraded in a system. Since pH was also found to be a factor in experiments which utilized fermentation products, the pH was regulated to maintain a neutral environment for the production and use of fermentation products. The two main aspects of the experiment would be to (1) see if pH regulation aided in the uptake of fermentation products (i.e. fewer remain at the end of the cycle) and (2) quantify the effect of retention on cellulose degradation.

Materials and Methods

3.1 Inocula and Medium

The fuel cells were inoculated with either a binary culture of *Geobacter sulfurreducens* (5 mL) and *Clostridium cellulolyticum* (5 mL), grown in their respective ATCC medium and then inoculated directly into the fuel cells, or an undefined mixed culture using sludge obtained from the Penn State University sewage treatment facility secondary clarifier (10 mL).

The growth medium used in all the fuel cells was that described by Ren et al. (2007), and the recipe is included in Appendix A. It was comprised of 100 mM phosphate buffer with trace minerals and vitamins, and MN301, a purified form of insoluble amorphous and microcrystalline cellulose (Macherey-Nagel, Duren, Germany), as the electron donor. Medium was de-oxygenated using N_2 sparging for 1 hour prior to being autoclaved. The buffer portion of the medium was made and autoclaved separately in 160 mL anaerobic bottles to avoid precipitation. Each medium was made at twice the final concentration of components. Filter-sterilized vitamins and L-cysteine (0.5 g/L final concentrations) were added to the medium in the anaerobic chamber after autoclaving. The later runs in this experiment did not use cysteine in order to evaluate whether the community voltage production was dependent on the addition of cysteine to the reactor.

3.2 Reactors

The MFCs utilized were single-chamber bottle reactors (Figure 3-1) with a platinum-catalyzed air cathode as described by Logan et. al. (2007). The carbon-cloth cathode was 11.3 cm^2 in exposed area and had a platinum loading of 0.5 mg/cm^2 and a PTFE diffusion layer (Cheng et al. 2005). No membrane resided between the anode chamber and the cathode. The anode was a 2 cm by 6 cm piece of carbon paper connected with epoxy to a 24 gauge copper wire. Resistance for the anode (which was found to be <6 Ω for all anodes) was tested to make sure good connections were achieved in each reactor. The volume of the medium in the reactors was approximately 280 mL, although 290 mL was used in the start up run. This allowed for the anode to be completely submerged in each reactor, while leaving headspace in the bottle reactor for anticipated gas production. An anaerobic stopper was embedded in the top of the fuel cell caps to allow for pH and headspace sampling.

Figure **3-1**: Bottle MFC reactor used in these experiments

3.3 Operation

The reactors were started with a volume of 290 mL (medium + inoculum). Three reactors were run for each inoculum. This included one reactor that was completely drained after each run, one that had a portion of the anode-chamber contents retained, and one that was operated on cysteine only (no cellulose) as a control. The fuel cells were operated in fed-batch mode with a feeding schedule of every 10 days. For each feeding, the MFCs were transferred into an anaerobic chamber. A sample was taken from each fuel cell of the spent medium while well mixed. Then, one MFC was completely emptied and fresh medium was added. The L-cysteine control was also operated in this manner. In the carryover MFC, approximately 50 mL of well-mixed anode-chamber contents was

retained. This carryover from the previous run gave the reactors a slightly longer solids reaction time (SRT) and allowed for the retention of microorganisms that might be more associated with the cellulose particles than the anode. Reactors were stirred constantly at a low rpm, and periodically tipped to obtain samples for pH measurements and to resuspend particles that may have settled. During the run the cells were mixed to facilitate the transfer of cellulose degradation products to the exoelectrogens on the anode.

Initial experiments (referred to as Experiment 1) used the same cultures and medium described above, but did not have routine pH and gas measurements measured for the duration of that trial. They were operated in the same type of feeding schedule, but were only refed when the voltage reached below 20 mV. The other difference in operation was that the cell with carryover for each inoculum was settled (i.e. it retained all solids) before being decant to leave approximately 50 mL in the anode chamber. The reactors themselves were exactly the same, but new anodes and cathodes were used for the second set of experiments with full data sets.

3.4 Measurements

3.4.1 Voltage Power Production

The voltage of the cell was measured over a 1000 Ω resistor using the Pico Meter for the first experiments, and then a Keithley Model 2700 DMM, Data Acquisition,

Datalogging System in the second set of experiments. Both dataloggers read the voltage of each cell every 30 minutes. Power was then calculated using the Eq. 3.1

$$P = \frac{V^2}{R} \qquad \textbf{3.1}$$

Power density was calculated in terms of projected anode surface area (0.00024 m^2).

3.4.2 Coulombic Efficiency

Coulombic efficiency (CE) was calculated on the basis of the change in Total COD using Eq. 3.2 (Rabaey 2005; Cheng 2006).

$$C_E = \frac{8\int_0^{t_b} I\, dt}{Fv_{An}\,\Delta COD} \qquad \textbf{3.2}$$

where 8 is the molecular weight of oxygen divided by the number of electrons required to reduce molecular oxygen to water (32/4), the integral is the integrated current over time (the charge transferred over a given period of time (0 to t_b)), F is Faraday's constant which converts charge (in coulombs) to electron equivalents, v_{An} is the volume of liquid in the anode chamber, and ΔCOD is the difference in the total COD over that designated time period (Logan 2008).

3.4.3 COD Measurements

COD was measured using HACH low range (0-150 mg/L) test-in-tube vials or Chemtreck mid-range (0-1500 mg/L) test vials. Sample dilutions (20X for the HACH vials and 4X for the Chemtreck vials) were introduced into the vials in 2 mL increments, digested, and then read according to the manufacturer's directions. Samples were taken at both the beginning and the end of the feeding cycles and preserved with sulfuric acid and stored at 4^{o}C until the subsequent analysis. Each sample was evaluated for three constituents: total COD, soluble COD, and COD due to cellulose. Cellulose was isolated using the washing protocol stated in section 3.4.6 and tested for COD at a dilution of 20X and 4X (HACH and Chemtreck vials, respectively). For soluble COD, samples were pre-filtered using a 0.2 um pore syringe filter. After reacting for 2 hours in a 150^{o}C block, the vials were read in the HACH 2800 Spectrophotometer at the specified wavelengths for each vial type. For the low-range vials, standard curves were developed using COD standards of 150, 100, 50, 25 and 0 mg/L. The mid-range vials had standards of 1000, 500, 250, 100, and 0 mg/L. Each sample was run in triplicate.

3.4.4 Internal Resistance

The internal resistance was estimated using polarization curves, which are graphs of voltage versus current density over variable external resistances from 20 Ω to 10.7 MΩ. The internal resistance was estimated by assuming that the R_{int} was the slope of the polarization curve shown in Eq. 3.3. Curves identifying the resistance values selected for these calculatinos are shown in Appendix A.

$$V = R_{int} I + OCV \quad \textbf{3.3}$$

3.5.5 Gas Composition

The gas composition in the headspace of the reactors was analyzed using a SRI 310C gas chromatograph (GC) with PeakSimple 3.29 software with a six inch Restek column using argon as the carrying gas. Samples were taken in 500 μL gas tight syringes and peak areas were compared to standards to obtain actual concentrations. Standards were done by injecting different volumes of laboratory air (100, 200, 300, 400, and 500 μL), pure hydrogen, pure nitrogen, and pure methane at atmospheric pressure into the GC. The headspace was measured at the beginning and end of a cycle. The gases that were analyzed were hydrogen, oxygen, nitrogen, and methane. The residence time was used to delineate between the gasses with hydrogen's peak appearing first, then respective peaks for oxygen, then nitrogen, then methane. Hydrogen and methane were of particular interest because they represented possible electron sinks in the reaction.

3.5.6 Cellulose Degradation

Cellulose concentration was measured by first washing the cells from the cellulose. This was done by pelleting the sample (using centrifugation at 6000g for 8 minutes), removing the supernatant, resuspending the pellet in PBS, centrifuging (6000g for 8 minutes), removing the supernatant, resuspending in 1M NaOH, then centrifuging (6000g for 8 minutes), and removing the supernatant (Ren, Ward et al. 2007). The

cellulose pellet was then dissolved in 10 mL of 67% sulfuric acid overnight at 4^{o}C (Updegraff 1969). This product was then added in 2 mL increments to spectrophotometric tubes, in which the colorimetric determination of glucose was carried out. This method included the addition of 50 μL of 80% phenol then mixing, followed by the addition of 5 mL of 96% sulfuric acid. Test tubes were then shaken and placed in a 28^{o}C water bath for 15 minutes. The asorbance was then measured in a spectrophotometer at 490 nm (Dubois 1956). The cellulose was quantified by measuring it against standards that had been prepared from pure MN301 (100 mg/L, 50 mg/L, 20 mg/L, 10 mg/L) in triplicate that went through the same preparation (including biomass washing to incorporate possible losses). This procedure was carried out in triplicate for each of the 4 dilutions done for each cell (10X, 20X, 50X, 100X), since this method has been shown to be accurate for concentrations of 10-100 mg/L.

3.5.7 Volatile Fatty Acids

The fuel cells were analyzed for volatile fatty acids (VFAs) using high performance liquid chromatography (HPLC) in the Waters 2695 Separations Module HPLC. Samples were filtered through 0.2 μm supor filters, and 1 mL of each sample was placed into 2 mL glass liquid chromatography vials and clamped with a Teflon-lined aluminum septum. One cycle was analyzed for each fuel cell, with samples collected before and after the chosen cycle. Standards were run for each of the VFAs analyzed at concentrations of 10, 5, 1, 0.5, and 0.1 mM.

3.5.8 Quantitative PCR

Quantitative PCR (polymerase chain reaction) was done on the binary MFCs to monitor the time-series abundance of each population, the exoelectrogen *G. sulfurreducens* and the cellulolytic fermenters *C. celluloyticum*, and to compare the populations between fuel cells with and without carryover. In preparation for quantitative PCR, suspension samples were frozen after each run. DNA extractions were then carried out using a PowerSoil DNA kit and the protocol provided.

Prior to this experiment, primers were designed by Lisa Steinberg to quantify *C. cellulolyticum* and *G. sulfurreducens* abundance in MFC suspensions using 16S rDNA. The primer pair targeting *C. cellulolyticum* and other members of *Clostridium* Group III consisted of forward primer Clos956f (5'-CCTTCTGTGCCGGAGTTAACA-3') and reverse primer Clos1428r (5'-CCCACMATCTGAACTGGGACTAT-3'), which generated a fragment of approximately 470 bp. The primer pair targeting *G. sulfurreducens* and other closely related *Geobacter* consisted of forward primer Geo587f (5'-GGGAGGGAAGAAATGATTG-3') and reverse primer Geo978r (5'-GGGCTCAATACCCGCCAACA-3'), which generated a fragment of approximately 400 bp. Primer sequences were checked for specificity using the Probe Match function of the Ribosomal Database Project II release 9 (http://rdp.cme.msu.edu/) and the BLAST function of GenBank (http://www.ncbi.nlm.nih.gov/Genbank/index.html), and they were checked for appropriate melting temperature and possible secondary structures using NetPrimer (http://www.premierbiosoft.com/netprimer/index.html). Experimental primer verification was performed with DNA extracted from cultures of *C. cellulolyticum* and *G.*

sulfurreducens. PCR conditions consisted of an initial denaturation at 95°C for 3 min followed by 30 cycles of denaturation at 95°C for 30s, annealing at 54°C for 45s, and extension at 72°C for 30s, with a final extension at 72°C for 7 min. PCR products were run on a 1% agarose gel at 15 V/cm for 25 min to confirm appropriate size and no non-specific amplification. Fragments from this PCR were ligated into vector pCR2.1 and cloned into *E. coli* Top10 cells using a TA cloning kit (Invitrogen, Carlsbad, CA) following the manufacturer's instructions. Blue-white screening was used to determine clones containing the PCR fragments. Positive clones for each PCR product were grown overnight at 37°C in LB broth containing 100 µg/mL ampicillin. Plasmids containing the PCR fragment insert were purified using a QIAprep Miniprep Kit (QIAGEN, Valencia, CA). Plasmid was quantified at 260 nm on a spectrophotometer, and stock solutions of 10 ng/µl were made in 10 mM Tris-HCl, pH 8.5. Standard curves for real-time quantitative PCR were prepared in 10 mM Tris-HCl, pH 8.5 as 10-fold dilutions from 1 ng/µl to 0.002 pg/µl. Quantitative PCR was performed on a Bio-Rad iCycler using 96-well polypropylene plates and optical sealing tape (Bio-Rad, Hercules, CA). Standards were run in duplicate and samples from the MFCs were run in triplicate (at 1x and 10X dilutions). Total reaction volumes of 20 µl contained final concentrations of 1X PCR buffer and .03 U/µl Taq polymerase (USB, Cleveland, OH), 2.5 mM $MgCl_2$, 0.25 µM each primer, 0.5 M Betaine, 10 nM fluoroscein (Bio-Rad, Hercules, CA), a 150,000x dilution of SYBR Green (Molecular Probes, Invitrogen, Carlsbad, CA), and 2 µl of template DNA. Reaction conditions were the same as already described except that 40 cycles of PCR were run and each cycle contained a 10 s step at 85°C inserted after

extension for image capture. This step was necessary to eliminate fluorescence caused by the formation of primer dimers during the PCR (Ren et al. 2008).

Results

4.1 Voltage Production and pH

In experiment 1, in which all solids were retained in the carryover reactors by settling and decant, the sludge-inoculated reactor that was settled prior to decant and re-feeding produced less peak and overall power throughout these cycles than the similarly inoculated reactor that had no carryover (Figure 4-1). In the carryover reactor, the voltage peaked and then decreased over the next few days, and the peak power within each subsequent cycle was reduced. The average run length was about 12-14 days, with the medium being replaced after the voltage had dropped to < 20 V. There was a definite lag within the system to initiate power production, which was not exhibited in the later experiments. The only time in which almost immediate maximum power generation occurred was in the cycle beginning at time 190 hours, where the medium of the no carryover cell was replaced prior to significant voltage drop. This lag may have been a symptom of oxygen accumulation in the reactors as they operated at depleted substrate levels. The cellulose degradation of these cells was not quantified, but the medium after cessation of power generation was still cloudy and white with what appeared to be residual cellulose.

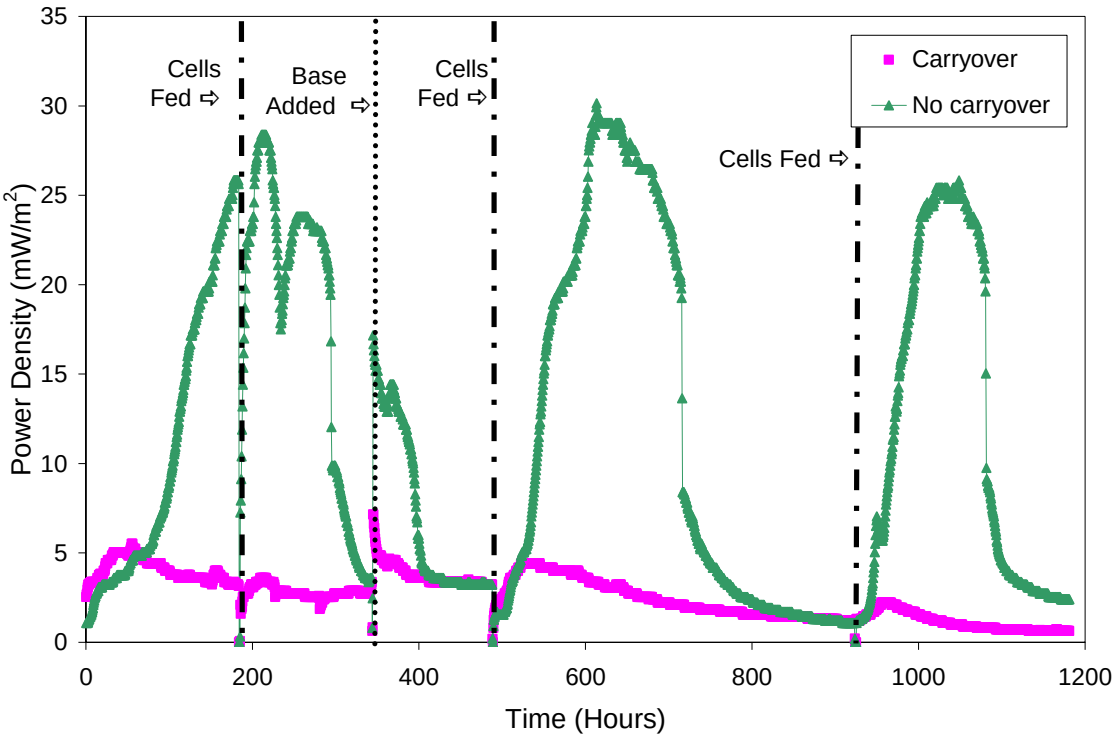

Figure **4-1**: Sludge-inoculated MFC power density profiles for experiment 1

Unlike with the sludge-inoculated reactors, carryover in the binary system had the effects of increasing the voltage production and decreasing the start up period (Figure 4-2). The voltage production in the MFC without settled material appeared to be more erratic than the cell with carryover.

The wire of the anodes in this first experiment's reactors broke during operation of these systems prior to complete analysis of cellulose conversion, headspace gas composition, and community dynamics. This experiment was terminated, and experiment 2 was initiated with the slight modification in carryover procedure, run time, and the attempt to keep pH above 6.0.

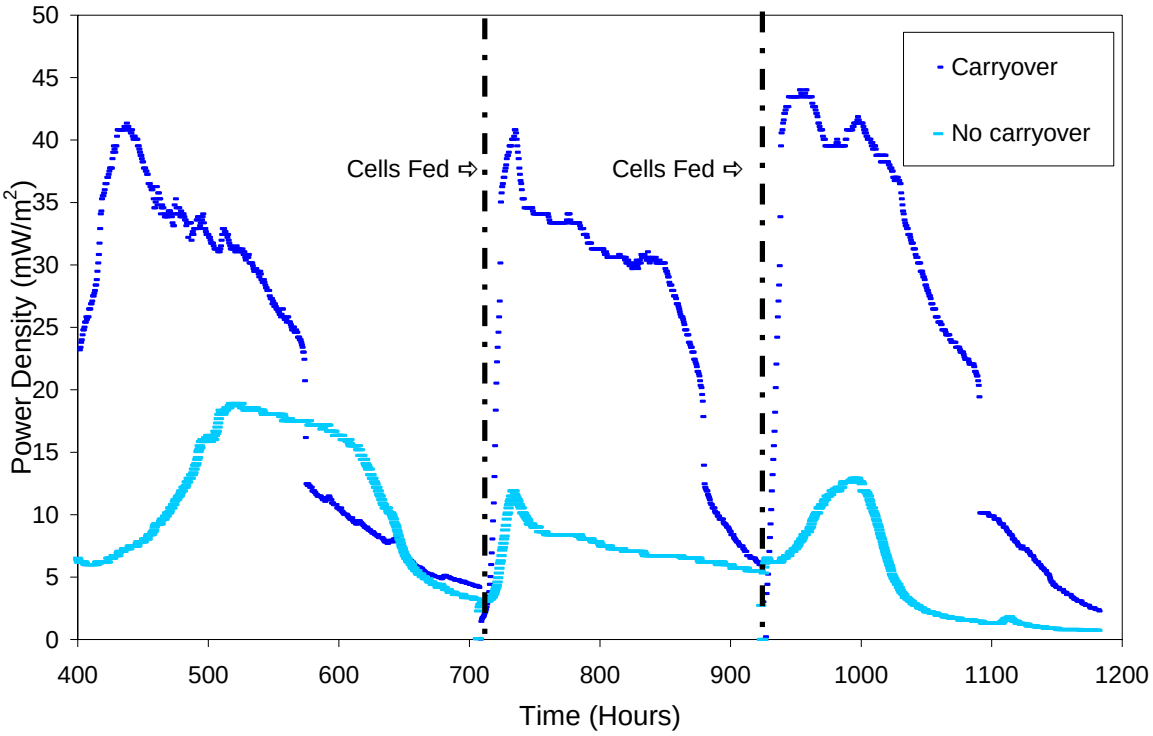

Figure **4-2**: Binary-inoculated MFC power density profiles for experiment 1

The results from the second set of experiments differed from the first set in several ways. There was not a constant power production in the binary-inoculated MFCs (Figure 4-3), and carryover did not enhance power production. Less power was produced in the binary reactor with carryover than the system without carryover. Carryover was observed to induce rapid drops in pH. With the sludge-inoculated fuel cells (Figure 4-4), the reactor without carryover initially produced more power than the system with carryover, as seen in experiment 1. However, with repeated cycles and the removal of l-cysteine from the medium, this system converged on the same reduced power level. As in the binary systems, the sludge-inoculated reactor with carryover had more rapid declines in pH and needed more base addition to maintain the pH above 6.0.

When cysteine was removed from the feed at 77 days, both systems continued to produce comparable amounts of electricity. In all systems, a voltage drop appeared to correlate with many of the pH drops, and sharp increases in power production occurred after the addition of base (1 M NaOH). More pH corrections were required with the MFCs having carryover, meaning the pH in these cells dropped more quickly than in those cells without carryover, although pH increases without base addition can be seen as well (any increase in pH where the line is not vertical). There was little to no difference in the lag time of the reactors. Once medium was added to the fuel cells, all cells (except for those with cysteine only) immediately began to produce power.

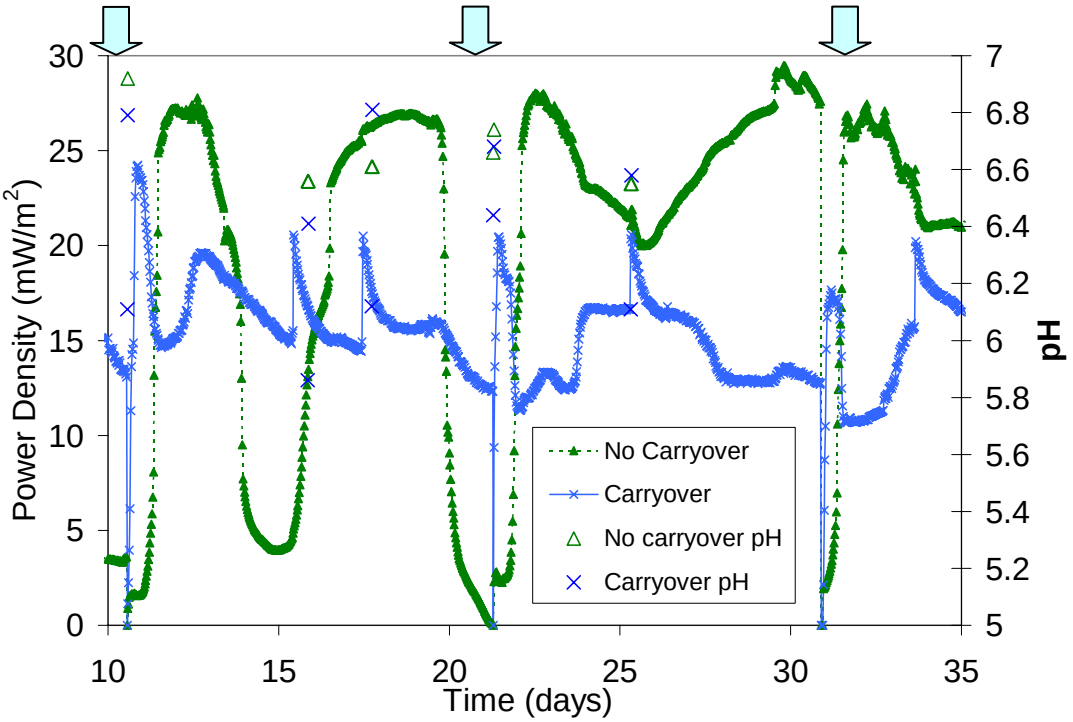

Figure **4-3**: Power density profile and pH of binary-culture MFCs, arrows denote feedings. Fully evaluated cycle was between 11 and 22 days.

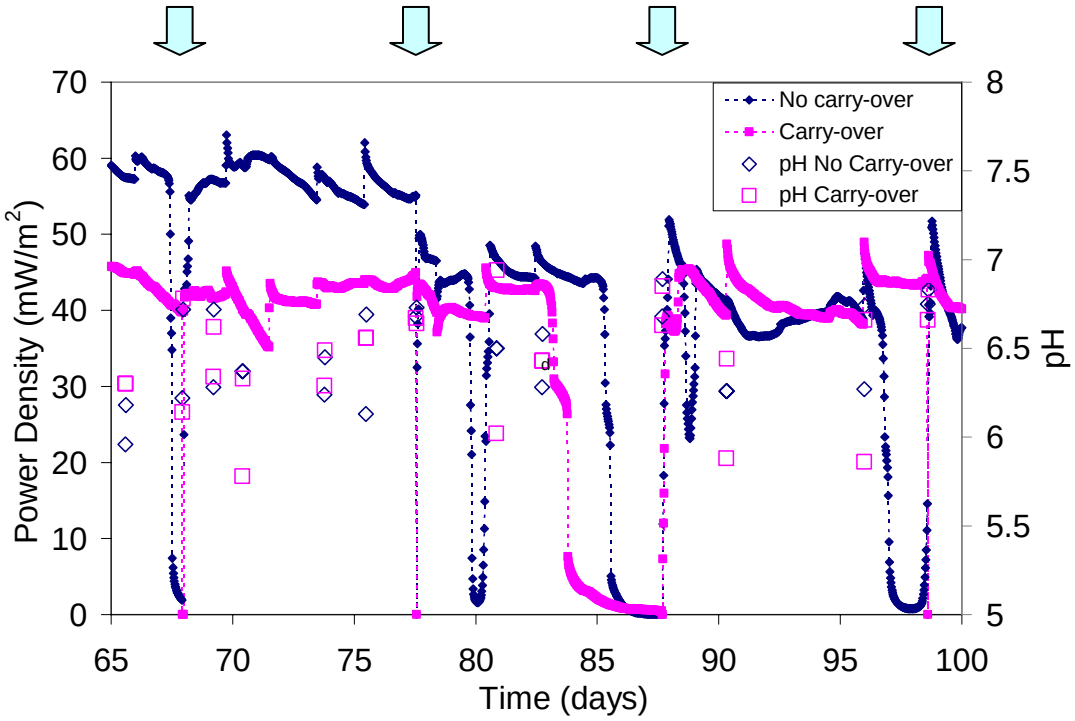

Figure **4-4**: Power density profile and pH of sludge MFCs, arrows denote feedings. Fully evaluated cycle was between 68 and 78 days.

4.2 COD Removal and Coulombic Efficiency

The COD standard curves for both vial types were linear across the range of respective standard concentrations, showing R^2 values of 0.9986 (binary cells using 0-1500 mg/L Chemtreck COD analysis vials at a 4X dilution) and 0.989 (sludge-inoculated MFCs using HACH 0-150 mg/L vials at a 20X dilution). Coulombic efficiency was calculated based on the cumulative current and the change in total COD (Table 4-1), as described before. The data is shown in mg, not mg/L, because the concentrations were

corrected for the total volume differences (due to evaporation, addition of base, etc.) that occurred from the beginning to the end of a run.

Table **4-1**: COD removal and Coulombic Efficiency for selected MFC runs

Inoculum	MFC	COD start (mg)	COD end (mg)	ΔCOD (mg)	% COD change	Coulombic Efficiency (%)
Binary	Carryover	847	356	490	58%	3.1%
	No carryover	888	764	124	14%	11.4%
Sludge	Carryover	726	202	524	72%	4.2%
	No carryover	639	205	434	68%	5.8%

4.3 Internal Resistance and Maximum Power Density

Internal resistance was estimated utilizing polarization curves, which were constructed by recording the voltage (once stable) over a range of resistances from 50 Ω to 10.7 MΩ. For each resistance, the average voltage produced was calculated and then the power and current densities were calculated for each average voltage. This was done for all four of the cells (Figure 4-5 for the binary curves and Figure 4-6 for the sludge MFC curves). The internal resistances were lower for the fuel cells that were sludge-inoculated, and the corresponding maximum power densities were higher in these cells. For both inocula, the systems without carryover produced higher power densities; this was more pronounced in the binary systems (Table 4-2). The curves (V vs. I) are identified in Appendix A (Figure A-1).

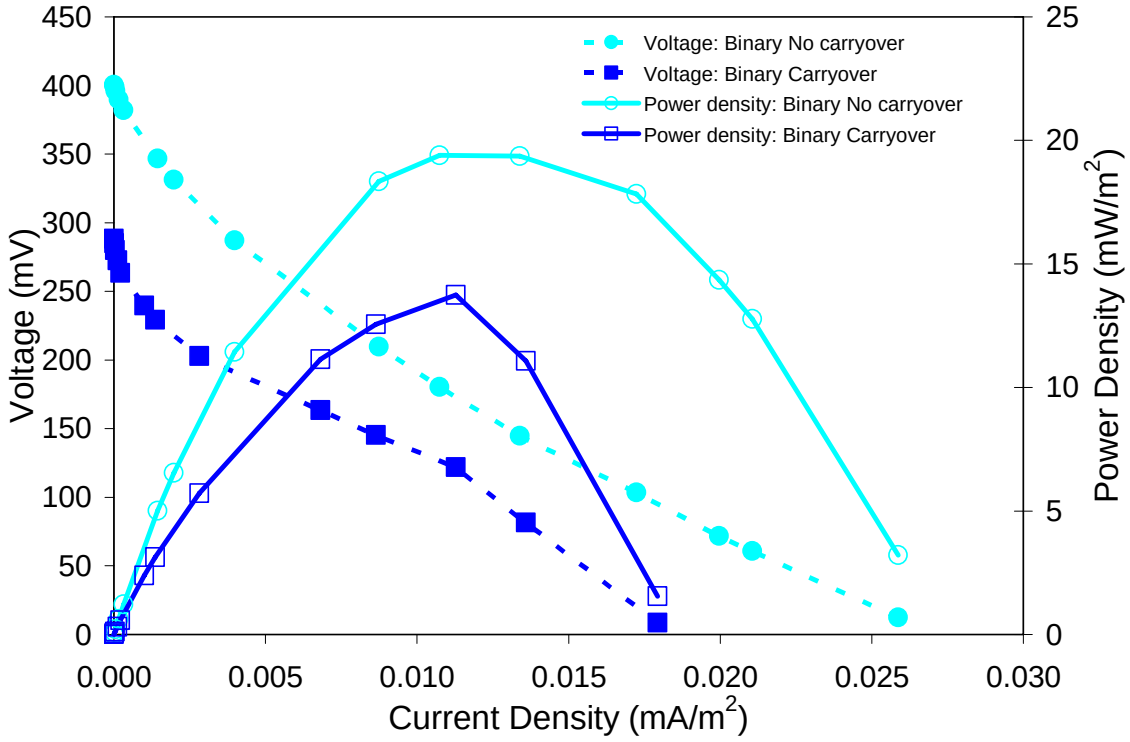

Figure **4-5**: Power density curves for binary-inoculated MFCs

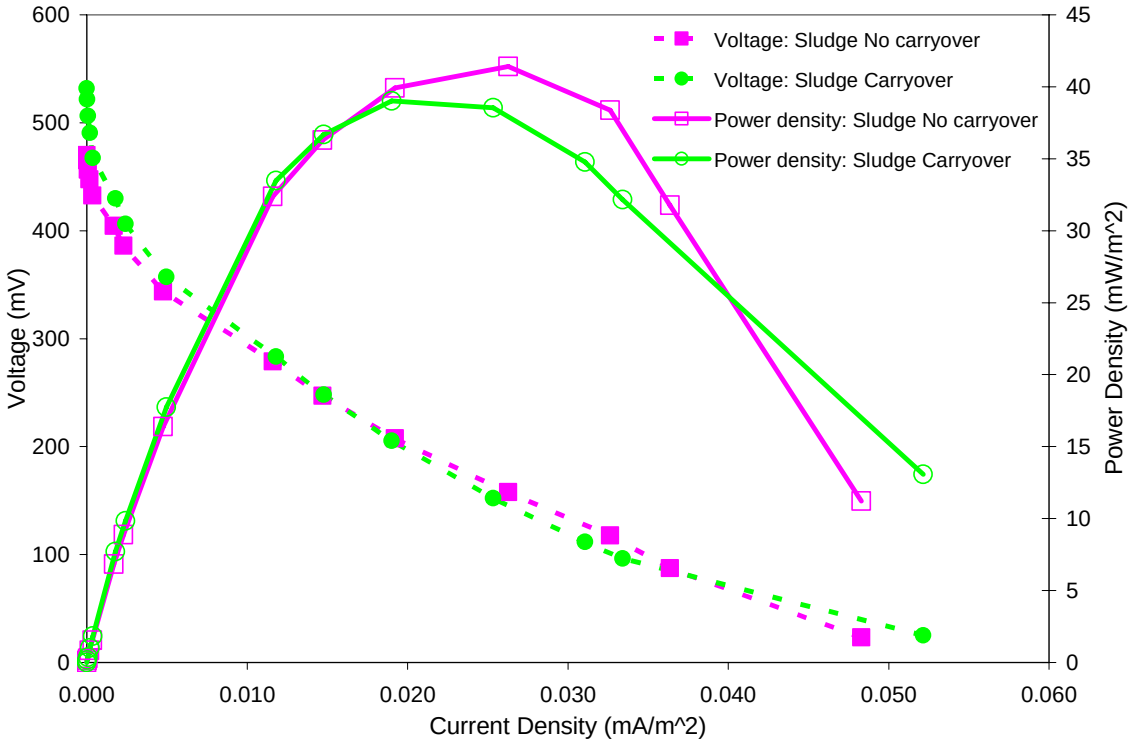

Figure **4-6**: Power density curves for sludge-inoculated MFCs

Table **4-2**: Internal resistances and maximum power densities of MFCs

MFC	Internal Resistance (Ω)	Maximum Power Density (mW/m^2)
Binary - No Carryover	593	19.4
Binary - Carryover	485	13.7
Sludge - No Carryover	330	41.4
Sludge - Carryover	393	39.0

4.4 Gas Production

Headspace gas composition was measured right before the end of each cycle in the sludge-inoculated reactors. Methane was only detected in the system with carryover

(Figure 4-7); no methane was detected in the sludge-inoculated MFC without carryover. Gas production was not monitored in the binary MFCs due to inability for sterile gas sampling and since neither *G. sulfurreducens* nor *C. cellulolyticum* is able to produce methane. At time 88 days, the MFC was resealed due to concerns that gas may have been leaking out of the cell, as seen by oxygen in the cell during the previous run. No hydrogen was detected in the sludge-inoculated reactors. Nitrogen fluctuations appeared to be negligible.

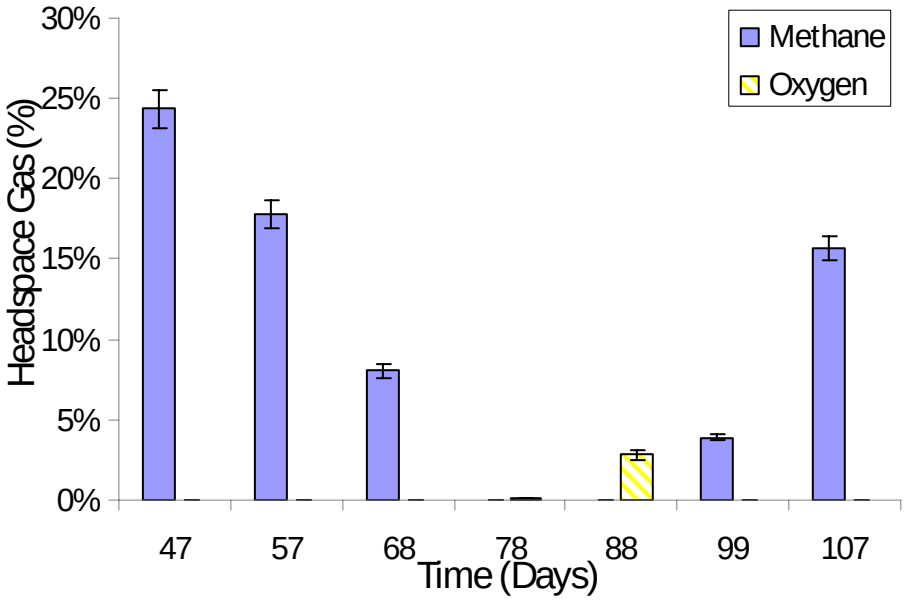

Figure **4-7**: Methane and oxygen in the sludge-inoculated carryover MFC

4.5 Cellulose Degradation

The cellulose measurements showed that more degradation occurred within the sludge-inoculated fuel cells, and cellulose degradation was significantly enhanced by

operating with carryover (Table 4-3). This analysis was also done using COD analysis of samples that had the biomass removed through alkaline lysis and washing, as described in the cellulose section of the materials and methods. The results, from these two approaches, were comparable.

Table **4-3**: Cellulose degradation on mass basis

Inoculum	**MFC**	**% Cellulose Converted**	**% Cellulose Converted (COD basis)**
Binary	With carryover	90%	92%
	Without carryover	31%	33%
Sludge	With carryover	95%	94%
	Without carryover	56%	56%

4.6 VFA Analysis

Based on the HPLC data, considerable amounts of VFAs remained after the run, which led to high initial VFAs in those reactors having carryover (Table 4-6). A negative value in the Δ row denotes a reduction in the VFA concentration throughout the run, and the total column in this row represents the change in the concentration of all measured VFAs over the course of the run. Percentage increase/decrease was not calculated due to the fact that many acids were not present initially in the feedstock. All concentrations were less than 5 mM, except for the final acetic acid concentration in the binary MFC with carryover, which had a higher concentration of acetic acid.

Table **4-4**: VFA concentrations over one run

			VFA (mM)								
	Inoculum	**MFC***	**formic**	**acetic**	**propionic**	**2-methyl-propionic**	**butyric**	**3-methyl-butyric**	**valeric**	**hexanoic**	**Total**
Start of Run	Binary	A	0.4	0	0.6	0	0	0	0	0	1.1
		B	0	1.1	0.5	0	0	0	0	0.4	2.1
	Sludge	A	0.3	0	0.2	0	0	0.1	0.2	0	0.8
		B	0.1	8.3	0.5	0.3	0.6	1.7	0.3	0.1	11.8
End of Run	Binary	A	0.2	0	0	0	0	0	0.1	0.2	0.5
		B	0	1.1	0.5	0	0	0	0	0.4	2.1
	Sludge	A	0.2	0	-0.5	0	0	0.1	0.2	0	0
		B	0.1	7.2	-0.1	0.3	0.6	1.7	0.3	0.1	10.1
Δ	Binary	A	-0.2	0	-0.6	0	0	0	0.1	0.2	-0.5
		B	0	0	0	0	0	0	0	0	0
	Sludge	A	0.4	0	0.6	0	0	0	0	0	1.1
		B	0	1.1	0.5	0	0	0	0	0.4	2.1
*MFC A is without carryover and B is with carryover											

4.7 Quantitative PCR

Based on the standard curve, the linear dynamic range for qPCR for *Clostridium cellulolyticum* was seven orders of magnitude, from 415 to 4.15×10^8 target gene copies/reaction. The reaction efficiency was 86.9%, with a Y-intercept of 39.9 and R^2 of 0.9983. Template controls did not show amplification for these reactions. The number of *C. cellulolyticum* 16S rDNA genes in suspension at the end of each cycle (copies/mL of medium) are chronologically shown in Figure 4-8. The cell without carryover had two values (at 11 and 32 days) that were below the standard curve generated, i.e. less than 415 genes were found. These two data points remain on the figure for the purposes of visualizing the difference between the two MFCs. The presence of the fermenters in the no carryover MFC suspension was greater than 0, but only slightly. A table of these values can be found in Appendix A.

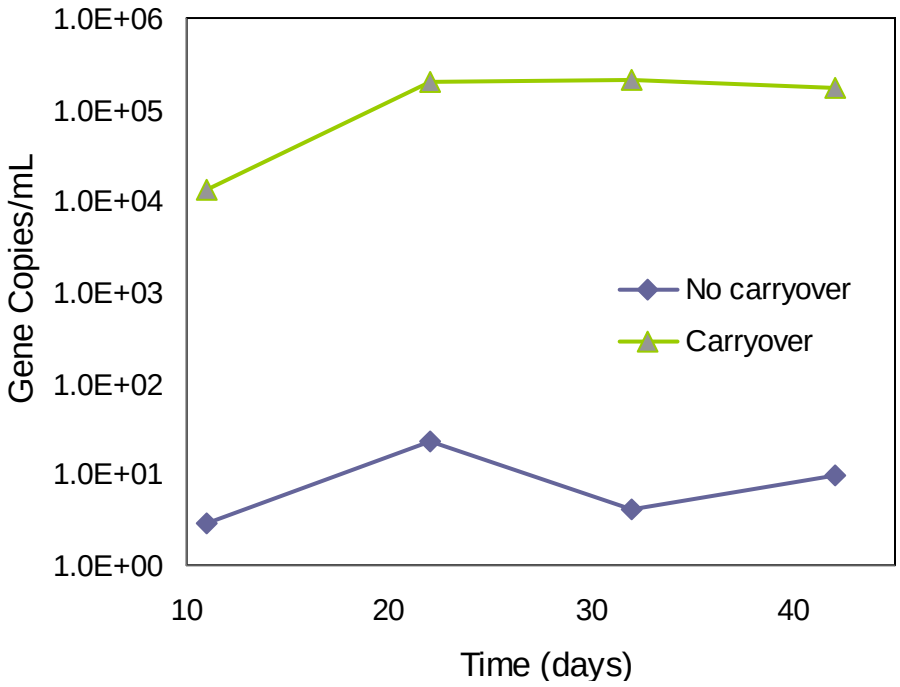

Figure **4-8**: Prevalence of suspended *Clostridium cellulolyticum* in binary MFC over 4 cycles

For the *Geobacter sulfurreducens* standard curve, the linear dynamic range for qPCR was seven orders of magnitude, from 422 to 4.22×10^8 target gene copies/reaction. The reaction efficiency was 108%, with a Y-intercept of 34.3 and an R^2 of 0.9801. Template controls did show amplification for these reactions, with water blanks showing detection of PCR products after 29.6 cycles. The number of *G. sulfurreducens* 16S rDNA genes present in suspension at the end of each cycle (copies/mL) are chronologically shown in Figure 4-9. There appeared to be an increase in the suspended *G. sulfurreducens* concentration over time for both cells, but the cell without carryover appeared to decrease in concentration over the last run.

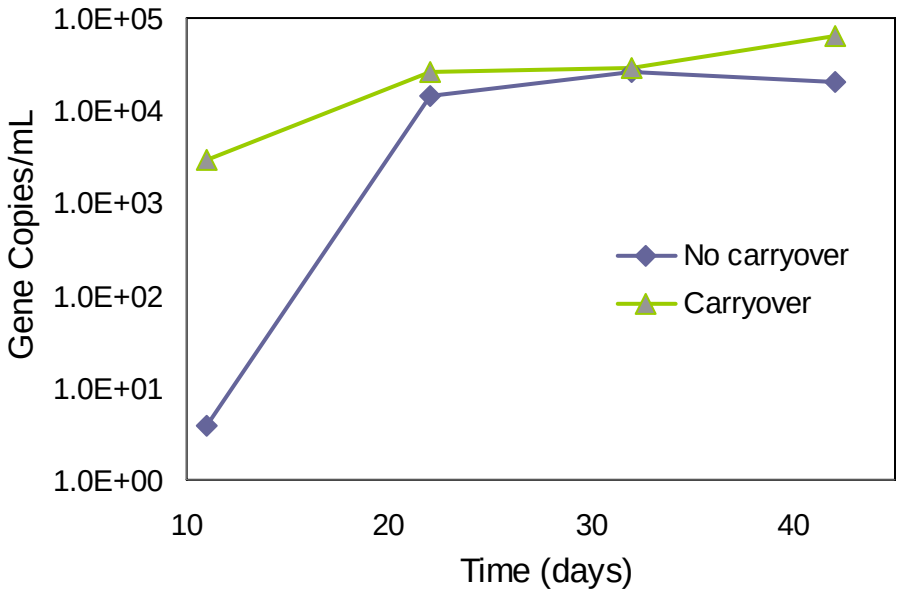

Figure **4-9**: Prevalence of suspended *Geobacter sulfurreducens* in binary MFC over 4 cycles

Discussion

5.1 Power Production Limitations

The pH has been found to be a determining factor in the amount of cellulose broken down and subsequent power generation in other experiments (Ren, Ward et al. 2007; Ishii, Shimoyama et al. 2008). This was also found in these experiments, as power production was reduced as the pH fell below 6. Adjusting the pH led to increases in power production; the voltage increased immediately after addition of base and then declined as pH decreased again. This pH drop with cellulose conversion was not always observed. It appeared to be most problematic in the cells with carryover, and particularly in the binary culture. The VFA results indicate that the exoelectrogens in the carryover reactor were less able to metabolize fermentation products relative to their rates of production.

Oxygen infiltration appeared to be a major problem with keeping the fuel cells running at a constant level of power production. This could have been a main factor in the longer run time of 10 days or more compared to small cubic reactors, which had run times of 1-2 days. Some decreases in power production could only be rectified by transferring the cells into an anaerobic chamber. Some of the sludge-inoculated MFCs' earlier instability could be related to the cysteine being used up and the conversion of cellulose into power being delayed by the inability of the cysteine-fueled population to

adequately hydrolyze cellulose and further process it for the exoelectrogens to use in exo-electron generation. Also, the depletion of cysteine would allow oxygen (which should be scavenged by the cysteine) to diffuse through the air cathode and accumulate in the liquid. Oxygen infiltration is a main concern, especially in the binary reactors because *G. sulfurreducens* is not aerotolerant at high levels of oxygen concentration in the headspace (15-20%) and could be intolerant of even smaller amounts of oxygen if the media itself was not anaerobic (Lin et al. 2003). A schematic of how the binary cultures work with each other and how oxygen entered the cell is shown below (Figure 5-1).

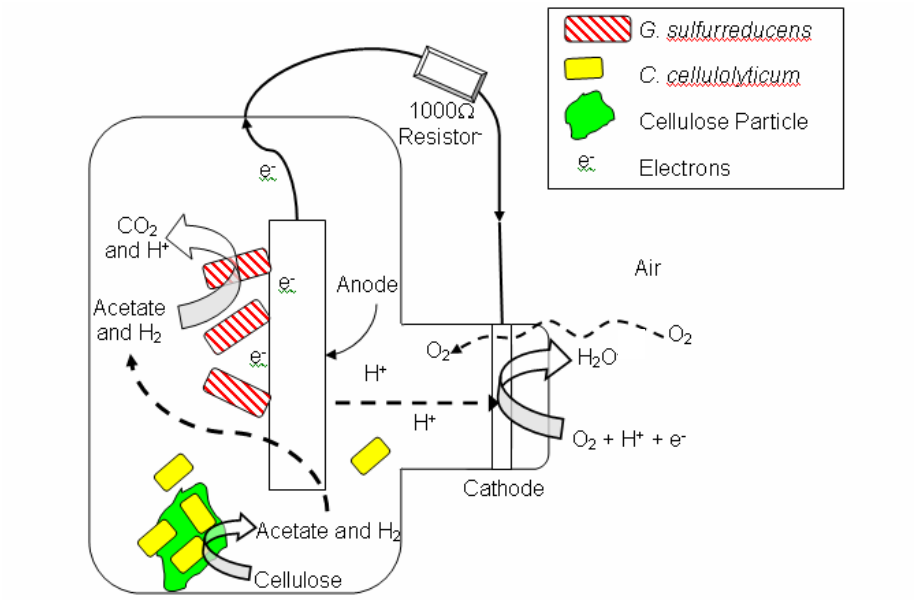

Figure **5-1**: Schematic of binary MFC interactions and oxygen infiltration.

The drop in the power production of the sludge MFC without carryover to the same level as the sludge MFC with carryover could be due to the fact that cysteine was purposefully left out of the cell starting with the run beginning at day 78 (Figure 4-4). This then proved to limit the amount of power produced by the cell to that of the cell with

carryover, which could be related to any of the issues above. A power drop was not seen in the cell with carryover, perhaps due to residual cysteine or a more robust aero-tolerant microbial community.

The control reactor for each experiment was operated without the addition of an electron source besides cysteine. There was no significant power generation by the binary control reactor. This inability of *C. cellulolyticum* and *G. sulfurreducens* to generate electricity from cycteine was also shown in a two-chamber MFC with the same binary inoculum in experiments done by Ren et. al. (2007).

The sludge-inoculated control MFC showed a significant power density profile (data not shown), but the power production at a resistance of 1000 Ω was less than the cells with cellulose and cysteine. Also, there was no significant drop in power once the cysteine was removed from the medium (time 78 hours). Power production also ceased in the control cell two days before the ten day run time had expired. The power generation for the control cell also exhibited lag in the start up of its power production. This same phenomenon was not seen in the fuel cells using cellulose. The power density in this cell was comparable (~45 mW/m^2, not optimized) with that reported in other studies looking only at cysteine as a fuel for MFCs (Logan, Murano et al. 2005). That study reported that the power density of the MFC fuel cell fed only cysteine to be 33 mW/m^2. Logan et al. (2005) also discovered that the power density was related to the concentration of cysteine in the medium (0.5 g/L for the experiments in this thesis, 0.385 g/L for Logan et al. (2005)). This control reactor only had a coulombic efficiency of 5.1% (based on filtered COD measurements), but it had a COD removal efficiency of 89%. This could be due to cysteine being used up by infiltrating oxygen molecules in the

air cathode system and also biomass formation since the MFC had small brown flocs in it at the end of the cycle, as well as a biofilm on the glass after the second cycle.

In experiment 1, the power profiles of sludge-inoculated MFCs with no settling looked similar to the cysteine control in experiment 2. The lower power production with the first inoculation could be explained by cellulose-degrading organisms not being present at the levels necessary to break down and hydrolyze the complex. This theory does not explain why one MFC produced power and the other produced little if any, unless the few exoelectrogens in the cell with carryover were out competed by other community members. The start up period for experiment 1 (not using cysteine) was much longer and less stable than the start up period (the time directly following inoculation) for experiment 2 (utilizing cysteine).

5.2 Power Density and Internal Resistance

Even with the loss of electrons to methane and biomass, the performance of the mixed culture was superior to that of the binary fuel cells. This phenomenom of mixed cultures out-preforming defined cultures has been exhibited in similar fuel cells comparing mixed inocula and pure cultures (Logan 2006; Logan 2008; Ren 2008). Although some previous work had shown more power production from binary systems (Ren et al. 2007), that study used dissimilar electron acceptors (ferric cyanide for the binary culture, oxygen for the mixed culture) so the values could not be compared.

The power densities exhibited in experiment 2 of this present study fluctuated from cycle to cycle in the binary fuel cells, with the start-up cycle actually producing more

power than the subsequent cycles. Experiment 1 exhibited a maximum power density for the binary fuel cell with carryover more than two times larger than that of the second experiment (~44 mW/m^2 compared to 19 mW/m^2).

The internal resistances of the binary MFCs were also higher than those of the sludge MFCs. All the internal resistances were more than 5X higher than values reported in a study looking at glucose in a bottle air-cathode MFC with carbon paper anode, which found an internal resistance of only 65 Ω (Logan et al. 2007). This could be due in part to biofilm growth of non exoelectrogens on conductive surfaces and the mixing of the medium in the fuel cells. The maximum power densities were not high once runs had more or less stabilized for power production.

5.3 Gas Production

In the first experiment, reduction in the power output of the sludge-inoculated cells may have been due to the holdover of methanogens in the retentate. The gas sampling in the latter experiment explored this possibility and found it to be a viable explanation, as methane was indeed found in the fuel cells. This increased reaction time (by the carryover of some substrate) is a good operating practice for anaerobic digestion, but for MFCs to be feasible, the electrons must not be transferred to methane, but rather operated so as to capitalize on the ability of the microorganisms' exoelectron transferring capabilities. The shorter periods of electricity production could be explained by methanogenesis taking place in the cell after greater holdover time. The stop in methane production could have been due to the seal becoming worn after several runs, leading to

methane escape and oxygen intrusion and the rise in methane production later could be explained by resealing the fuel cells at day 88. The two prior runs to this showed no methane production, but did show oxygen intrusion (Figure 4.5).

5.4 COD and Cellulose Removal

The quantification of cellulose and COD showed interesting patterns, as the greatest amount of cellulose degradation occurred in the sludge-inoculated MFC. A loss of about 95% of the fed cellulose was shown. In the work done by Ren et al. (2007) cellulose degradations of 40% (sludge-inoculated) to 49% (binary inoculum) were observed. The studies by Ishii et al. (2008) , Rismani-Yazdi et al. (2007), and Rezaei et al. (2007) did not report findings for cellulose removal. For this experiment, the least amount of cellulose degraded was in the binary MFC without carryover. This cell also had the highest coulombic efficiency, which again implied that there might be hydrolysis limiting this MFC. The no carryover sludge-inoculated MFC also had low amounts of VFAs present, implying that it too may have a hydrolysis issue in breaking down this complex substrate into useable monomers, rather than a lack of substrate for the exoelectrogenic community.

5.5 Volatile Fatty Acids

The HPLC data showed that VFA concentration existed both before and after the runs. The small amounts of VFAs prior to the run in the medium could be attributed to

several things. One possible source for VFAs would be the yeast extract in the medium. Another source could be the slight break down of cellulose in the sterilization (autoclaving) of the medium. Also, the communities in the MFCs began to form biofilms (which were more prevalent in the sludge-inoculated MFCs), which may have harbored some VFAs from one run to the next. The fuel cells were not rinsed, nor cleaned in any way, prior to the next run. The structure of the fuel cell with the protrusion out of the body of the bottle for the air cathode (Figure 3-1) lent to the collection of cellulose particles there because of the substrate being continuously mixed. The cellulose collected in this area was difficult to resuspend due to the cellulose being partially attached to the cathode in the case of the sludge MFCs. This slime, as well as the biofilm that had a tendency to form over the glass around day 77, showed that there may be issues with clogging due to the insoluble form of cellulose.

5.6 Real-time PCR

Quantitative PCR showed that the carryover of the substrate results in an accumulation of cellulose-degrading organisms in the binary MFCs. The MFC without carryover had little to no quantification of fermenters (below standards used), which could explain the irregularity of the power production peaks in the MFCs. This data supplemented with the HPLC data and the cellulose degradation data, lends one to believe that the rate-limiting step in these “no carryover” reactors is hydrolysis of the cellulose itself, possibly due to the lack of organisms capable of this conversion in the binary MFC. VFA concentrations (especially acetate) were much higher in the cells with

carryover. The incorporation of the old medium (which should contain some cellulose and VFAs) would increase this concentration due to the VFAs already present within this feedstock. The presence of that concentration of acetate might serve to inhibit some biological processes, and would definitely result in the lowered pH exhibited by the binary MFC with carryover.

5.7 Future work

In the future it would be interesting to look at the pH of cellulose-fed MFCs over a run with different concentrations of cellulose in the feedstock. This may be able to show if the cells are able to "recover" after large drops in pH due to the production of organic acids from high rates of organic loading, as was seen on the small scale in this work.

Due to the influx of oxygen over time, a more effective strategy might be operating the reactor as a flow through reactor instead of mixed fed-batch systems. This would still facilitate mixing of the components without the introduction of oxygen, as well as being a more realistic reactor design.

Also, it would be interesting to look at the methanogen numbers in the fuel cells, to see if they are absent in the MFC without carryover, or if they are there and just out competed. Another interesting use of quantitative PCR would be to apply what was done in this report to the sludge inoculum. A wider array of primers would be investigate fermenter and exoeletrogen diversity.

Using a more realistic feedstock, i.e. that has lignin and hemicellulose as components, to see how these different biomass constituents affect these reactions would be an engaging experiment. The complexity of real biomass may have difficulties in the MFC environment, but may be more realistic. It would also be interesting to see if this technology could be linked to pretreatment of biomass, such as with an acid to dissolve the hemicellulose and cellulose and run at acidic conditions (perhaps with an acidophilic exoelectrogen) to obtain electricity directly from the dissolved biomass in acid, without conversion to ethanol or some other intermediate.

6

Conclusions

Based on the operation and analysis of binary and sludge-inoculated cellulose-fed air cathode MFCs, the following conclusions were drawn:

1) The effects of carryover in the performance of cellulose fed MFCs serves to both increase cellulose degradation and COD removal without adverse affect to power generation, with a consistently buffered pH.
2) MFCs without carryover appeared to have greater coulombic efficiency, but due to lack of cellulose degradation, appeared to be limited by hydrolysis in performance.
3) The second experiment showed no effect of carryover on the amount of time required for the systems to begin producing power.
4) There appeared to be a trade-off (more power in exchange for less cellulose conversion) in carryover effects. This trend was more apparent in the binary reactors than in the sludge-inoculated reactors.
5) Although cysteine is a viable electron donor and carbon source for sludge microbial consortia, it proved useful in starting of an MFC to eliminate oxygen infiltration during start-up.

Appendix A

A.1 Medium (Ren et al. 2007)

1.05 g NH_4Cl

1.5 g KH_2PO_4

2.2 g K_2HPO_4

2.9 g $MgCl_2 \cdot 3H_2O$

2.9 g $CaCl_2 \cdot 3H_2O$

0.1 g Bacto$^{(R)}$ yeast extract

10 mL trace minerals

Medium made at concentrations of 2X per L.

Degassed with N_2, transferred into an anaerobic chamber where it was dispensed into 160 mL anaerobic bottles in either 140 mL (for the no carryover) or 115 mL (carryover) aliguots.

MN301 was added to the minerals (not the phosphate portion of the medium) at a mass of 0.56 mg which would allow for a final concentration in the cell of 2g/L.

The bottles were then sealed, crimped, autoclaved and stored until feeding. Prior to feeding, L-cysteine (0.14 g) and 280 mL of 10X vitamins were combined, filter sterilized and then added to the medium.

Minerals(usually 5-10X)

	g/L
NTA	1.5
$MgSO_4$	3
$MnSO_4 \cdot H_2O$	0.5
NaCl	1
$FeSO_4 \cdot 7H_2O$	0.1
$CaCl_2 \cdot 2H_2O$	0.1
$CoCl_2 \cdot 6H_2O$	0.1
$ZnCl_2$	0.13
$CuSO_4 \cdot 5H_2O$	0.01
$AlK(SO_4)_2 \cdot 12H_2O$	0.01
H_3BO_3	0.01
Na_2MoO_4	0.025
$NiCl_2 \cdot 6H_2O$	0.024
$Na_2WO_4 \cdot 2H_2O$	0.025
Vitamins (usually 5-10X)	
	mg/L
biotin	2
folic acid	2
pyridoxine HCl	10
riboflavin	5
thiamin	5
nicotinic acid	5
pantothenic acid	5
B-12	0.1
p-aminobenzoic acid	5
thioctic acid	5

A.2 Real-time PCR Supporting Information

Support for the Figure 4-1 is in Table A-1 e

Table **A-1**: *C. cellulolyticum* gene copies in binary MFCs

	Carryover		No carryover	
Time (days)	**Copies/mL**	**Copies/mL-sample**	**Copies/mL**	**Copies/mL-sample**
11	2.51E+05	1.32E+04	5.40E+01	2.84E+00
22	3.85E+06	2.03E+05	4.43E+02	2.33E+01
32	3.93E+06	2.07E+05	7.69E+01	4.05E+00
42	3.26E+06	1.72E+05	1.85E+02	9.74E+00

Highlighted values are below the standard curve for the analysis.

Support for the Figure 4-2 is in Table A-2

Table **A-2**: *G. sulfurreducens* gene copies in binary MFCs

	Carryover		No carryover	
Time (days)	**Copies/mL**	**Copies/mL-sample**	**Copies/mL**	**Copies/mL-sample**
11	5.47E+04	2.88E+03	7.39E+01	3.89E+00
22	4.94E+05	2.60E+04	2.75E+05	1.45E+04
32	5.45E+05	2.87E+04	4.94E+05	2.60E+04
42	1.22E+06	6.42E+04	3.87E+05	2.04E+04

Highlighted values are below the standard curve for the analysis, but amplified before the template control.

A.3 Polarization curves

Figure A-1 with identified points for internal resistance calculations.

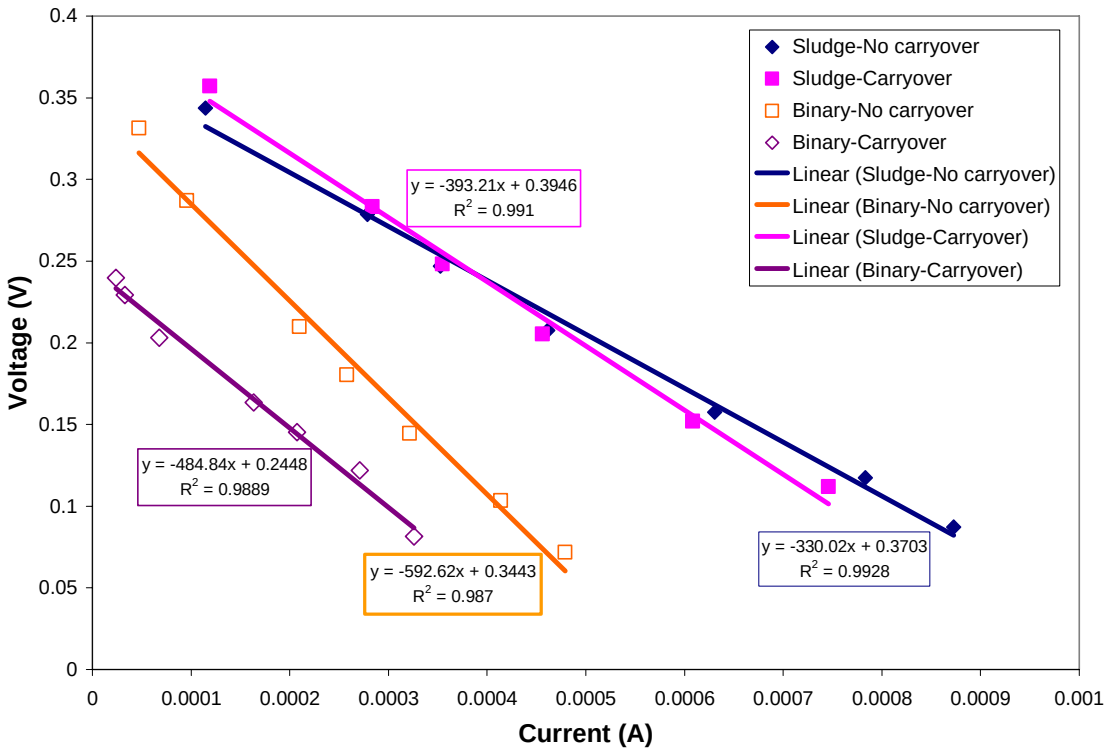

Figure **A-1**: Polarization curves with identified slopes for each reactor.

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