The Green Latrine: Development of a Large Scale Microbial Fuel Cell for the Treatment of Human Waste in Developing Areas

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A Masters Project Presented

by

Cynthia Castro

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Abstract

Global access to sanitation systems in the developing world has yet to be attained. Several concepts for small-scale ecological decentralized waste treatment systems have been developed to further disseminate sanitation systems in rural environments. These systems have added incentives such as compost and fertilizer obtained from human waste. The goal of this research is to develop a novel microbial fuel cell latrine that treats human waste and produces three incentives: treated effluent, compost, and electricity.

The MFC proposed in this work uses a simple three-chamber design. Each chamber is hydraulically partitioned, eliminating the need for a proton exchange membrane. A separate nitrification stage transforms ammonium, present in urine, to nitrate and a biocathode allows for nitrate removal. A pilot MFC was constructed and validated in the laboratory and deployed in Ghana.

Nitrogen and organic matter removal was observed during various operational conditions in Phase I before the MFC began treating synthetic feces and urine solutions during Phase II. During all of the operational conditions, COD removal was greater than 90%. Nitrate removal in Phase I reached up to 76.8 ± 7.1% while nitrogen removal during phase II was 68.4 ± 2.8 mg N/L. Power production reached an average 3.40 ± 0.01 nW/m^2 during the Phase I and decreased to 0.66 ± 0.02 nW/m^2 in Phase II. There was evidence of anaerobic digestion occurring in the anode, which limited power production by anode respiring bacteria.

The MFC latrine in Ghana was constructed in May 2012. Its performance is directly linked by its frequency of use. User interface challenges were observed.

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1.1 Introduction

There are 2.6 billion people in the world who lack access to basic sanitation, primarily in low and middle-income countries, including the majority of sub-Saharan Africa with sanitation accessible to less than 50% of the population (WHO/UNICEF JMP, 2013). Sanitation is necessary to prevent the spread of disease. In low-income countries, people lack basic sanitation infrastructure, which leads to open defecation and can contaminate open water bodies. These water bodies may be used for bathing and even as a drinking water source, exposing user groups to pathogens and viruses in waste. Children are often the most susceptible to these types of waterborne illnesses. Nearly 1.2 million children in low-income countries die each year due to diarrheal disease (UNICF, 2012)

1.2 Biological Degradation of Waste

Organic wastes and nutrients can be biologically degraded by microorganisms, mimicking transformation in natural systems. Biological treatment can occur in an aerobic or anaerobic environment. In both environments, microorganisms consume dissolved and colloidal organic matter present in wastewater. Heterotrophic bacteria can oxidize organic matter with oxygen as an electron acceptor in aerobic environments and electron acceptors such as sulfate, carbon dioxide, and nitrate in anaerobic environments (Rittmann & McCarty, 2001). Autotrophic microorganisms can also oxidize ammonium in aerobic environments. The aerobic and anaerobic microbial processes have been used

independently or as a combination for both centralized treatment and decentralized treatment to achieve the removal of organic and nitrogen wastes. In centralized treatment, wastewaters are collected and transported to a centralized location. Decentralized treatment occurs at the point of waste generation.

1.3 Centralized Wastewater Treatment Systems

Centralized wastewater treatment systems are commonly used in industrialized nations. A centralized wastewater system collects wastewater from homes and businesses, transports it to centralized treatment processing centers where constituents are removed from through physical, chemical, and biological processes, and the effluent and residual sludge are disposed of or reused (Tchobanoglous, Burton, Stensel, 2003) . Centralized systems are effective in economically advanced countries because they have the resources to support the infrastructure required to collect and treat wastewater. In developing countries, very few centralized wastewater treatment systems exist (Kivaisi, 2001).

The activated sludge process is the most widely used aerobic biological treatment method for municipal and industrial waste (Rittmann & McCarty, 2001). Microorganisms are “activated” by aeration provided to heterotrophic bacteria, allowing them to consume organic matter. Modifications of this process can achieve biological nitrification and the addition of anaerobic zones to the treatment train can facilitate denitrification and total nitrogen removal. Heterotrophic bacterial growth is significant and can produce large quantities of biomass, essentially creating a surplus of sludge wasted from the secondary clarifiers in centralized treatment systems.

Anaerobic biological processes are often incorporated into centralized treatment for the digestion of solid wastes. Anaerobic digestions is a complex multi-step process that occurs on the absence of oxygen that involves various microorganisms (Droste, 1997). The process entails three different steps: hydrolysis, acetogenesis, and methanogenesis. A group of microorganisms first hydrolyze complex organic matter into simpler carbohydrates, amino acids and fatty acids. Then, fermenting bacteria degrade simple carbohydrates and fatty acids into organic acids and hydrogen. The organic acids and hydrogen serve as electron donors for methanogens. Both anaerobic and aerobic treatment methods have their own advantages and disadvantages (Table 1). In large centralized wastewater treatment systems, the energy required for aeration can reach as much as 45-75% of the plant's total energy costs (Rosso, 2008).

Table 1. Comparison of anaerobic and aerobic biological treatment (Eckenfelder, 1988)

Parameter	Anaerobic	Aerobic
Energy Requirements	Low	High
Degree of Treatment	Moderate (60 to 90%)	High (95%+)
Sludge Production	Low	High
Process Stability (to toxic compounds & load changes)	Low to moderate	Moderate to high
Startup Time	2 to 4 months	2 to 4 weeks
Nutrient Requirements	Low	High for certain industrial wastes
Odor	Potential odor problems	Less opportunity for odors
Alkalinity Requirements	High for certain industrial wastes	Low
Biogas Production	Yes (net benefit is contingent on the need for reactor heating)	No

Currently, many wastewater collection systems are becoming more susceptible to contamination because they are aging rapidly. According to Moe and Rheingans (2006), the United States will need to invest over 6 billion dollars in pipe repairs within the next 25 years to maintain the infrastructure. There are over 600,000 miles of sewer pipes in the U.S., with over 35,000 breaks per year that cause the nation's piping system to function at less than 50% capacity (Tafuri & Selvakumar, 2002). Aging distribution systems are even more of a problem to developing countries with limited resources to repair the centralized infrastructure. Low and middle-income nations lack the funds and skilled labor to keep up with the demands of rapidly growing megacities and the need for adequate wastewater treatment, collection and distribution systems, and waste disposal will only continue to increase (Moe & Rheingans, 2006). Most of the population growth in megacities occurs within the cities slums, which are unlikely to be connected to water distribution systems or sewers. There are increased health risks to slum residents because there is inadequate excreta disposal, causing contamination of nearby water sources. Supporting centralized systems in rural areas of developing countries is impractical due to the lack of available sanitation infrastructure as well as high cost for installation and maintenance (Massoud, Tarhini, & Nasr, 2009). Therefore, alternatives to centralized wastewater treatment are required to meet the needs of the growing number of people without access to improved sanitation technologies. On-site, decentralized sanitation systems can provide long-term solutions because of their simple, low-cost designs, requiring minimal training and maintenance.

1.4 Decentralized Sanitation Systems

Many improved sanitation systems proposed for developing areas are decentralized. Improved sanitation facilities, as defined by the United Nations, are facilities that ensure separation between humans and their excreta (WHO/UNICEF JMP, 2013). These facilities include flush and pour-flush toilets that are connected to piped sewer system or septic tanks as well as ventilated improved pit (VIP) latrines and urine diverting composting toilets (Figure 1).

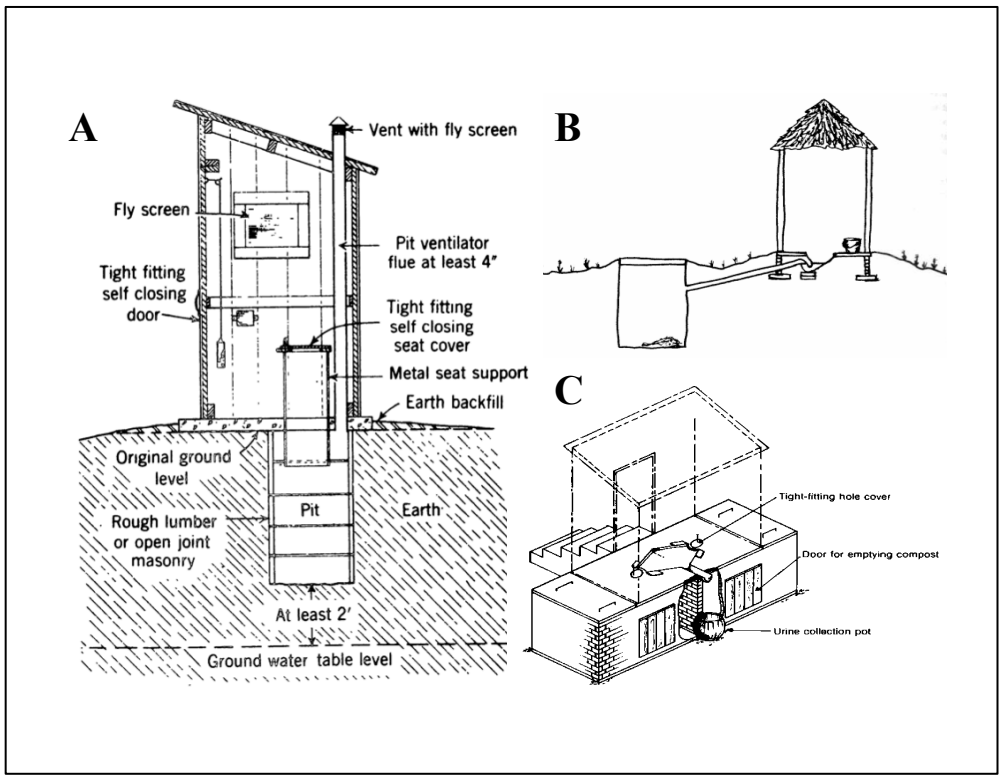

Figure 1. Improved sanitation facilities. A) Ventilated improved pit (VIP) latrine (World Bank, 1984); B) Pour-flush latrine connected to a septic tank (Mihelcic, 2009); C) Double vault composting latrine (Franceys, Pickford, & Reed, 1992)

These systems are not infallible. Seepage from pour flush toilets connected to septic tanks and pit latrines is known to cause contamination to groundwater and nearby surface waters (Esrey et al., 1998). In remote rural communities where no sewer systems

exist and access to domestic water sources is limited, dry pit latrines and composting latrines are adequate sanitation facilities because they require minimal maintenance.

1.4.1 Pit Latrines and Ventilated Improved Pit Latrines.

Sanitation infrastructure can be implemented successfully when the design takes into consideration social and cultural aspects of the communities. The simplest type of sanitation facility in the developing world is the traditional pit latrine. The traditional pit latrine consists of a hole in the ground, a concrete slab covering an opening, and an enclosing structure (Mihelcic, 2009). The problems with these latrines are that they produce strong odors and they can quickly become breeding grounds for flies that can contaminate food, water, and people with feces. The VIP latrine is designed to address odor and fly infestation issues but it is more expensive than a traditional pit latrine since it requires vents and screen to control odor and flies (Esrey et al., 1998; World Bank, 1984). A vent is added to the pit below the latrine so that prevailing winds that enter the pit can aerate the waste and odors are transported out of a back vent (Figure 2). The vent pipes are painted black so that when they become heated by the sun, air can rise and pull odors out (Hazeltine & Bull, 2003). Since flies are strongly attracted to light, the vent contains screens so that flies are contained within the pit.

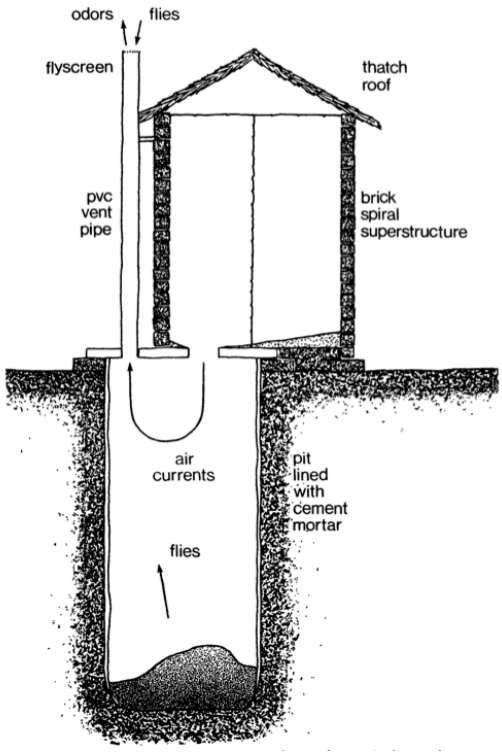

Figure 2. Prevailing winds through a VIP latrine (World Bank, 1984)

1.4.2 Ecological Sanitation and Composting Latrines.

Ecological sanitation takes a different approach to excreta disposal. Rather than viewing feces and urine as waste products, they are viewed as essential resources that can provide nutrients by recycling and reusing treated waste (Dellström Rosenquist, 2005). Ecological designs can range from decentralized wastewater treatment systems for communities to simple residential composting toilets. Ecological sanitation facilities have been implemented in various countries around the world including Mexico, El Salvador, Bolivia, Zimbabwe, South Africa, and India (Esrey, Andersson, Hillers, & Sawyer, 2001; Mnkeni & Austin, 2009; Moe & Izurieta, 2003; Ramani et al., 2012). For the purpose of this discussion, the focus will be on variations of small-scale ecological sanitation facilities like composting and urine diverting toilets.

Composting latrines typically fall under the umbrella of ecological sanitation (ecosan) facilities. Composting latrines not only provide a safe contained environment for excrement disposal and odor control, but can also make use of the waste to provide compost, fertilizer and biogas (Werner, Panesar, Rüd, & Olt, 2009). There are over a dozen different commercially available systems but most are intricately designed and unsuitable for low-income areas (Jenkins, 2005). The alternating double-vault composting latrine is an adaption of the VIP latrine. Solids are collected in one chamber, or 'vault' for a year. Once one of the compost chambers becomes full, the toilet seat is switched from one chamber to the other, and the waste is allowed to compost. After a year, the fully composted chamber is cleared out and can be used once again (Jenkins, 2005). In that manner, composting latrines have the potential to be used indefinitely. Another common design used in Zimbabwe is the arborloo designed by Peter Morgan (Morgan, 2007). The toilet is simple in design, where both urine and feces enter a shallow pit to prevent groundwater contamination and ash and soil are added after each use to reduce odors and fly breeding (Esrey et al., 2001). Before the pit is full, a tree is planted using the pit content and the superstructure is moved to a new site. VIP latrines are also non-urine diverting sanitation facilities frequently used in the developing world. Non-urine diverting latrines are adequate in areas where the water table is not high since seepage of waste can occur.

Like other biological wastewater treatment methods, excreta can be composted aerobically or anaerobically. Microorganisms use carbon as an energy source and also require macronutrients and trace elements for growth (Ryckeboer, Mergaert, Vaes, & Klammer, 2003). The carbon to nitrogen ratio within the compost is important to promote

growth. Human waste typically contains a carbon to nitrogen ratio of 5:1 and the ideal range for stable decomposition ranges between 15:1 to 30:1 (Esrey et al., 1998). Carbonaceous material such as sawdust and charcoal ash are added to increase the carbon source and as an absorbent material to control moisture content.

The various types of composting latrine designs consider different methods to control the composting temperatures and moisture content to optimize biological activity for degrading the organic matter. In aerobic decomposition, the rate of degradation is affected by the moisture content because the liquid provides a transport mechanism for nutrients to become accessible to microorganisms (Lopez Zavala & Funamizu, 2005). Zavala and Funamizu (2005) determined that a moisture content of 60% is optimal to enhance the rate of degradation. Aerobic decomposition occurs below the optimum moisture content while a combination of aerobic and anaerobic decomposition occurs above the optimum. Moisture content below 30% will cause microorganisms to become dormant while moisture content higher than 65% can cause oxygen depletion within the biofilms surrounding organic particles (Ryckeboer et al., 2003). Along with moisture content, composting temperatures also affect the biodegradation of the excreta. Heat is self produced within the compost matter by the microbial metabolic reactions that take place during the biodegradation process (Ryckeboer et al., 2003). The growth of microorganisms occurs between 0°C and 80°C, where composting is typically driven by mesophilic (10-35°C) or thermophilic (55-65°C) microorganisms (Lopez Zavala, Funamizu, & Takakuwa, 2004). Mesophilic composting can take a matter of months and can provide a humus substance usable for gardening or as topsoil. The higher temperatures achieved during thermophilic composting can deactivate disease-causing

organisms within the fecal matter and produce humus for food agriculture (Jenkins, 2005). Achieving the optimal temperature is critical in order to inhibit pathogen activation. Temperatures of 60°C and higher have been shown to effectively disinfect fecal matter in composting toilets (Lopez Zavala et al., 2004).

In some variations of composting latrines, rather than composting both urine and feces in one pit, urine is collected and diverted for other uses (Langergraber & Muellegger, 2005). Ecological sanitation toilets can be either urine diverting or non-urine diverting. Ecological sanitation toilets that use water for flushing are called urine diversion toilets (UDT) while dry units are called urine diverting dry toilets (UDDT). Whether urine is diverted or not depends on the method of feces treatment. The urine is collected through a urinal and a urinal pedestal (Figure 3). Urine is sterile within the body but once it is excreted, it can become contaminated with pathogens (C. L. Moe & Rheingans, 2006). The simplest method to destroy pathogens from urine is by storing it for at least six months at 20°C (Maurer, Pronk, & Larsen, 2006). Phosphorus and nitrogen can also be precipitated for nutrient recovery. Collected urine can be used directly on soil as a fertilizer or it can be diluted one to three with water when used on plants to prevent scorching (Esrey et al., 1998). For urine-diverting toilets, composting chambers are designed to either desiccate, increase the pH, or increase temperatures of the compost to kill pathogens (Esrey et al., 2001).

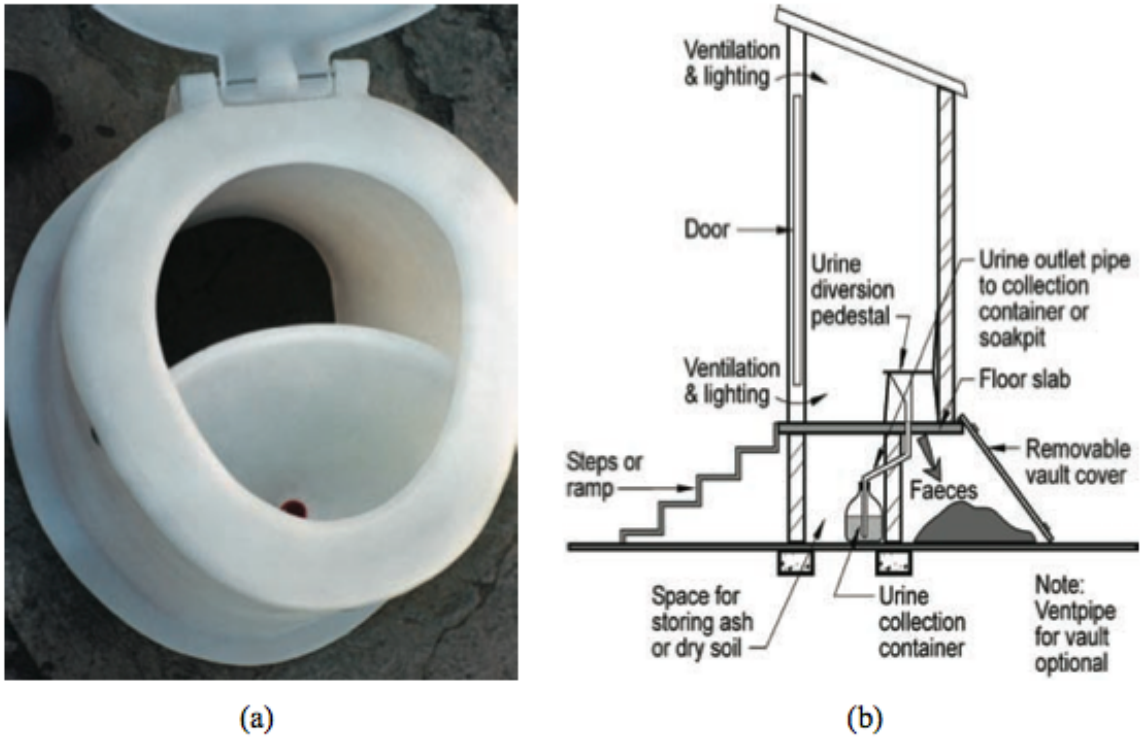

Figure 3. (a) Urine diversion pedestal (b) Schematic of urine-diversion toilet (Mnkeni & Austin, 2009)

1.4.3 Biogas Toilets

As an alternative to composting solid wastes, anaerobic digestion of organic wastes can produce biogases such as methane. Methane can be used as a source of fuel for cooking, heating or lighting and the waste digestate can be used as fertilizer (Hessami, Christensen, & Gani, 1996). In context, biogas has been produced in many parts of China for nearly a century (Chen, Yang, Sweeney, & Feng, 2010). According to Chen et al. (2010), biogas digesters in southern China have been coupled with pigpen and toilet waste to produce renewable energy. Very few studies have been conducted on biogas toilets (i.e. anaerobic digesters that solely use human waste from toilets) but several projects have been completed in India and Kenya. These systems consist of shallow pits where human

excreta are deposited and the waste flows into the digester where anaerobic digestion takes place to produce biogas (Schouten & Mathenge, 2010). Although biogas toilets produce renewable energy, they are not feasible in all locations of the developing world. Social challenges exist where people do not accept the use of biogas toilets. They believe the gas produced from waste is unhygienic. Their personal religious beliefs may prevent them from using the gas for cooking.

1.5 Sanitation Development

The successful implementation of sanitation infrastructure is not only correlated with availability of the technology in low and middle-income countries but it is also highly dependent on the factors that affect people's decision making. Sanitation systems targeted for developing areas must consider household income, traditional and personal outlook on sanitation, and religious restriction to waste reuse. There have been many documented case studies of using ecological sanitation technologies to harvest compost, provide fertilizer, extract nutrients such as phosphorus and nitrogen, and produce biogas (Chen et al., 2010; Esrey et al., 2001; Langergraber & Muellegger, 2005; Mnkeni & Austin, 2009; Werner et al., 2009). The greatest factor that affects these systems from being successful worldwide is the user perception of sanitation and reusing human waste.

Social sanitation entrepreneurship is a concept developed by the early pioneers of sustainable decentralized sanitation systems who promoted their diffusion to improve sanitation and people's quality of life (Ramani et al., 2012). In India, major strides in alleviating poor sanitation were made by Dr. Bhindeshwar Pathak and Paul Calvert in the 1970s and late 1980s, respectively. Dr. Pathak designed a double vault composting toilet

where as Mr. Calvert designed a urine diverting toilet, suitable for areas in India where the water table was high and water logging due to high rainfall is common. These systems were engineered specifically for a facet of the Indian community and required more effort and education than simpler designs.

Ramani et al. (2012) suggests that for any sanitation entrepreneur to develop a technology in the developing world, several key steps should be undertaken before the implementation process. First, the entrepreneur must confirm that there is a need for the technology and that it is appropriate for the area in which it will be implemented. The failure of sanitation systems in the developing world is not due to the technology itself but rather the dissemination and appropriateness of that technology in a given area. India is a nation that has had success in promoting sanitation throughout the country after mass government lead approaches for implementation failed. By no means is the sanitation crisis in India solved, but sanitation entrepreneurs have alleviated it through proper venture movements.

To effectively deploy a sanitation technology, the organizing group or individual must confirm demand, determine delivery mechanism, and deliver final product. Educating the community on how the system works and building a prototype for the community to test is important in order to understand whether the technology will be accepted socially and become successfully diffused. Cairncross (2003) also suggests that many people desire sanitation facilities not for the reasons that we perceive to be most important. For example, non-profit organizations develop sanitation models to reduce the spread of disease, while people in the developing world have previously prioritized the aesthetic benefits of having latrines rather than the health benefits they can provide. A

more thorough understanding of human behavior and the social and economic drivers that impact people's decision making can aid in improving the global dissemination of sanitation technologies.

1.6 Sanitation in Ghana

Ghana has been a prospering country among the nations of Africa for the past 30 years since undergoing the Structural Adjustment Programs, sponsored by the World Bank and the International Monetary Fund (Konadu-Agyemang, 2000). This program is based on facilitating economic policies, international trade and finance to developing countries in an aim at reducing poverty and bridging the gap between the rich and poor (Riddell, 2013). Although these policy changes did promote economic growth and development in Ghana, national funds were allocated towards international trade and export and other internal economic strategies, while expenditures for social infrastructure were minimized (Konadu-Agyemang, 2000). Building conventional wastewater treatment systems like the few that are found in urban areas is unsustainable for smaller communities. It is estimated that the annual costs for this infrastructure are nearly 30 billion U.S dollars, excluding maintenance costs (Jewitt, 2011). From 1990 to 2010, improvements in urban sanitation have been steadily increasing but people are still practicing open defecation in rural environments (WHO/UNICEF JMP, 2012). Open defecation in rural Ghana has increased from 29% to 33% in the span of 20 years (Figure 4).

URBAN SANITATION Estimated coverage 2012 update				
Year	Improved	Shared	Other unimproved	Open defecation
1990	12%	44%	33%	11%
1995	14%	51%	25%	10%
2000	16%	59%	16%	9%
2005	17%	66%	9%	8%
2010	19%	73%	2%	6%

RURAL SANITATION Estimated coverage 2012 update				
Year	Improved	Shared	Other unimproved	Open defecation
1990	4%	20%	47%	29%
1995	5%	25%	40%	30%
2000	6%	31%	32%	31%
2005	7%	37%	24%	32%
2010	8%	43%	16%	33%

TOTAL SANITATION Estimated coverage 2012 update				
Year	Improved	Shared	Other unimproved	Open defecation
1990	7%	29%	42%	22%
1995	9%	35%	34%	22%
2000	10%	43%	26%	21%
2005	12%	51%	16%	21%
2010	14%	58%	9%	19%

Figure 4. Progress of urban, rural, and total sanitation in Ghana from 1990-2010 (WHO/UNICEF JMP, 2012a)

Several factors affect the access to sanitation facilities in rural environments, including limited financial resources, insufficient water, and lack of education concerning health and hygiene (Esrey et al., 1998; Massoud et al., 2009). A community study on household latrines in four rural communities in Ghana showed that dry sanitation technologies are the most viable because of limited water availability and the adaptability of the

technologies to the local environment (Keraita, Jensen, Konradsen, Akple, & Rheinländer, 2013).

Chapter 2: Microbial Fuel Cells

2.1 Overview and Applications

A microbial fuel cell (MFC) is an innovative biotechnology that uses microorganisms to oxidize organic matter and produce electrical energy (Figure 5). Just like a fuel cell, an MFC is divided into an anode and cathode. Microbial communities develop around an electrode surface in the anode and organic substrates are oxidized, transporting electrons from the anode electrode to the cathode electrode via electrical wiring, producing a current.

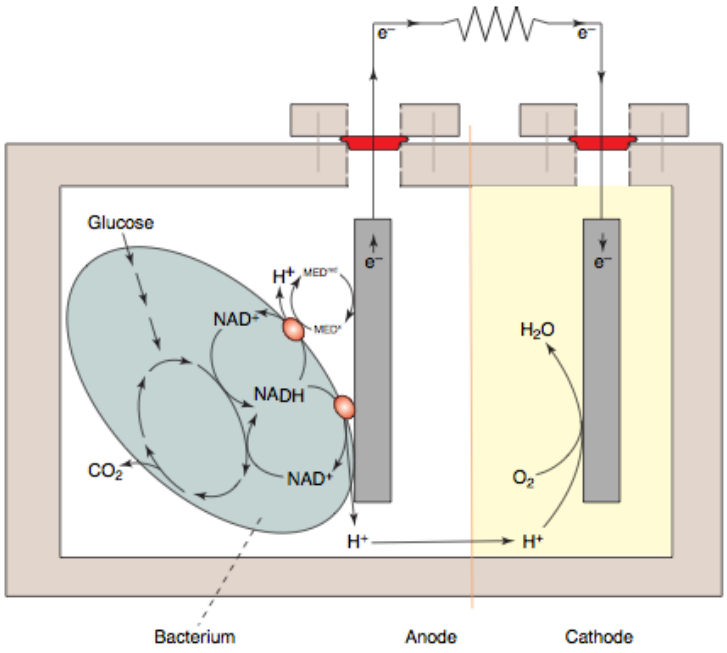

Figure 5. Simple microbial fuel cell designating how energy is produced via the oxidation of an organic substrate in the anode and oxygen as the electron acceptor in the cathode (Rabaey & Verstraete, 2005).

Bacteria in the anode have the capability of oxidizing a consortium of organic substrates such as acetate, glucose, starch, and waste sludges in an anaerobic environment. In the cathodes, electron acceptors can either be reduced chemically (abiotic cathodes) or biologically (biocathodes). Oxygen is the most predominantly used electron acceptor in the cathode, but requires oxygen-reducing catalysts such as platinum, pyroloyzed iron(II) phthalocyanine (pyr-FePc), hexacyanoferrate(III), and cobalt tetramethoxyphenylporphyrin (pyr-CoTMPP) to facilitate the reduction process (Cheng, Liu, & Logan, 2006; Zhao et al., 2006). Oxygen is widely used because of its abundance and ease of accessibility, and water molecules are obtained as the end product. Other electron acceptors have also been used for chemical cathodes such as ferricyanide and permanganate but they are unsustainable for long term use since they must be continuously regenerated (Park & Zeikus, 2003; You, Zhao, Zhang, Jiang, & Zhao, 2006). These systems are called abiotic cathodes. Biocathodes have also been developed that use microorganisms as biocatalysts to reduce electron acceptors (He & Angenent, 2006). More detail on biological cathodes is provided in the proceeding sections.

In two chamber MFCs, an ion exchange membrane separates the anode and cathode. The membrane prevents soluble electron acceptors from diffusing into the anode and allows ions to be transported between anode and cathode electrolytes. Ion exchange membranes are expensive and impractical for scaling up MFCs and studies have shown that they can reduce the overall MFC performance by increasing the internal resistance (Logan, 2008). New designs have used single chamber and two chamber membraneless MFCs, the latter producing 24.33 mW/m^3 with acetate as the electron donor in the anode and dissolved oxygen in the cathode (Du, Xie, et al., 2011; Jang et al., 2004). There are a

multitude of MFC designs that have been explored to enhance power production (Figure 6). MFC designs have used single chamber and two-chamber systems, membrane and membraneless reactors, simple organic substrates such as acetate and glucose as well as

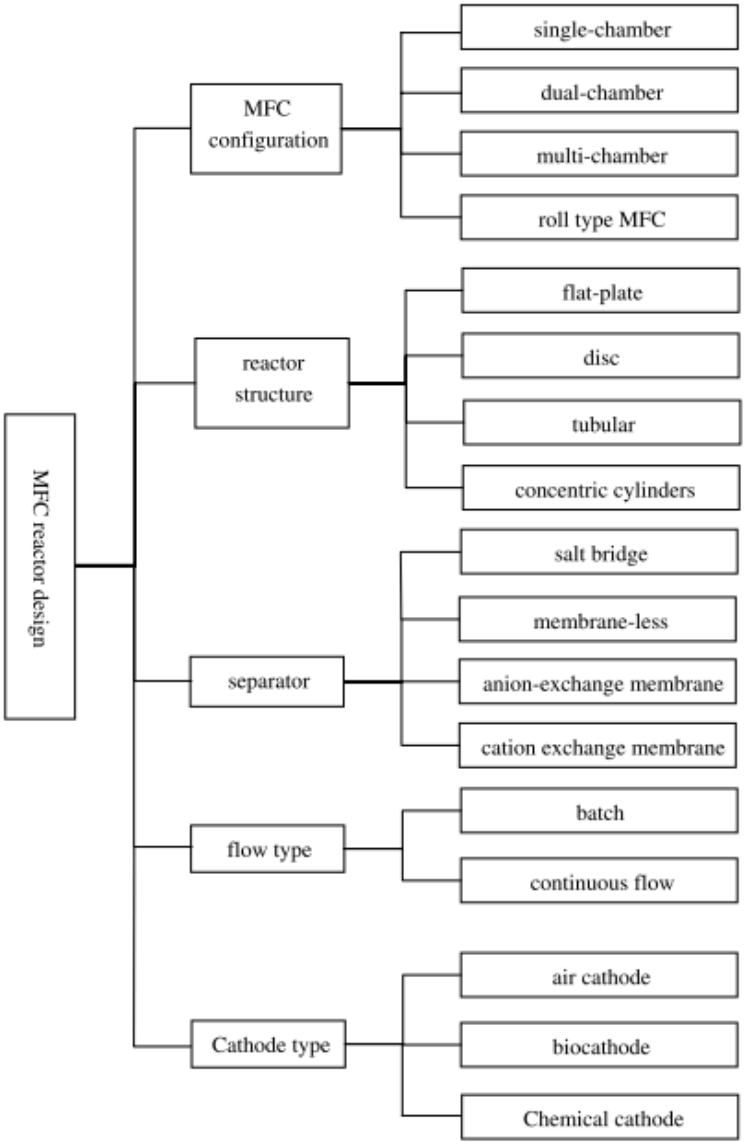

Figure 6. Classification of MFC reactors (Zhou, Wang, Hassett, & Gu, 2013)

domestic wastewaters, varying electrode materials, and abiotic cathodes and biocathodes (Harnisch & Schröder, 2010; He & Angenent, 2006; Liu & Logan, 2004; Pant, Van Bogaert, Diels, & Vanbroekhoven, 2010).

MFCs have been investigated to power biosensors and other small devices, as a wastewater treatment method, for desalination, and for the production of biohydrogen (Aelterman, Rabaey, Clauwaert, & Verstraete, 2006; Logan, 2008; Shantaram, Beyenal, Raajan, Veluchamy, & Lewandowski, 2005). According to several review articles, MFCs for large-scale energy production still face many challenges for real-world applications because of high costs and low power density output (Logan, 2010; Zhou, Wang, Hassett, & Gu, 2013). Most recently, development has been made instead on miniaturizing MFCs for medical applications, where enzymatic biofuel cells have been studied to power implantable medical devices (Wei & Liu, 2008; Yang, Ghobadian, Goodrich, Montazami, & Hashemi, 2013).

2.2 Anode-Respiring Bacteria

In 1910, Michael C. Potter discovered that electricity could be produced using yeast to degrade glucose in a precursor to a microbial fuel cell (Potter, 1911). In early studies of electrochemical bacteria, metal-reducing bacteria in aquatic sediments were discovered to use solid mineral oxides such as Fe(III) and Mn(IV) as their terminal electron acceptor during anaerobic respiration (Lovley, 2006). Solid electrodes made of graphite or platinum were introduced into anoxic marine sediments (anode) and connected to the aerobic seawater above (cathode) to see whether electricity could be produced via the reduction of a solid electrode (Bond & Lovley, 2003).

The earliest studies of MFCs that produced power used bacteria that required external additions of electron shuttles, or mediators, to transport electrons from inside the cell to the anode electrode (Chang et al., 2006). Microorganisms can also directly convey

electrons to an anode, using it as its terminal electron acceptor (Kim et al., 2002). Bacteria with this ability have been found in freshwater sediments, marine sediments, and wastewater treatment plants (Miceli, Parameswaran, Kang, Krajmalnik-Brown, & Torres, 2012). There are three main extracellular electron transfer (EET) mechanisms by which anode-respiring bacteria can transport electrons from the cell to the electrode surface. The first is by using soluble electron shuttles that are produced naturally by bacteria to transport electrons to the electrode (Lovley, 2006). Known electron shuttles produced by bacteria include melanin, phenazines, pyocyanin, flavins, and quinones (Rabaey, Boon, Höfte, & Verstraete, 2005; Torres et al., 2010).

The second mechanism requires direct contact of the outer membrane c-type cytochromes of a cell with the electrode to facilitate respiration (Lovley, 2006; Myers & Myers, 1992). Cytochrome c are heme-containing proteins that shuttle an electron in the electron transport chain (Alberts et al., 2004). Bacteria that use this mechanism with an insoluble electron acceptor cannot develop biofilms because they require direct contact with the electrode (Torres et al., 2010). The third mechanism involves cellular solid conductive or semi-conductive pili that act as nanowires to attach to the electrode surface and transport electrons from the cell (El-Naggar et al., 2010; Reguera et al., 2006). Several *Geobacter* lack c-type cytochromes but have been reported to complete respiration using the solid electrode through nanowires (Reguera et al., 2006). The nanowire electron transport mechanism is still unclear and theories have been developed on how this actually occurs. The first theory is the metal-like conductivity (MLC) hypothesis. It suggests that nanowires are metal-like conducting pili that use the attached cytochromes to transfer electrons from the nanowire to the solid electron acceptor

(Boesen & Nielsen, 2013; Malvankar et al., 2011). The second theory is the superexchange conductivity (SEC) hypothesis, which claims that electrons are transported by sequential electron-transfer self-exchange reactions between cofactors in a biofilm and that concentration gradients along the biofilm thickness drive this EET mechanism (Bond, Strycharz-Glaven, Tender, & Torres, 2012).

Table 1 contains a short list of known anode-respiring bacteria that have been used for MFC research. *Shewanella* and *Geobacter* have been extensively researched to further understand the mechanisms by which electrons are transferred to an anode.

Table 2. Anode respiring bacteria known to use extracellular electron transport to reduce solid electrodes

Microorganism	Electron Donor	Electrode Material	Source
Geobacter sulfurreducens	Acetate	Unpolished graphite	(Bond & Lovley, 2003)
Geobacter metallireducens	Benzoate	Graphite	(Bond, Holmes, Tender, & Lovley, 2002)
DesulfuroDemonas acetoxidans	Acetate	Graphite	(Bond et al., 2002)
Thermincola ferriacetica	Acetate	Graphite block	(Marshall & May, 2009)
Thermincola potens	Acetate	Graphite block & graphite carbon fiber	(Wrighton et al., 2011)
Shewanella putrefaciens	Lactate	Graphite felt	(Kim et al., 2002)
Shewanella oneidensis MR-1	Fumarate & Lactate	NA	(Lies et al., 2005)
Aeromonas hydrophila	Acetate	NA	(Pham et al., 2003)
Pseudomonas aeruginosa	Glucose	Graphite	(Rabaey, Boon, Siciliano, Verhaege, & Verstraete, 2004)
Rhodoferax ferrireducens	Glucose	graphite rod, foam and felt	(Chaudhuri & Lovley, 2003)
Clostridium butyricum	starch wastewater	graphite felt	(H. S. Park et al., 2001)

In bench scales studies, simple organics such as acetate and glucose have been used as the sole electron donor but various types of wastewaters such as domestic, swine

wastewater, and even sewage sludge have been used for power production (Min, Kim, Oh, Regan, & Logan, 2005; Pant et al., 2010; Zhang et al., 2012). Complex substrates in the anode allow for competition between anode-respiring bacteria and microorganisms that use the organic substrates for different metabolic pathways, such as acetogenesis and methanogensis (Oliveira, Simões, Melo, & Pinto, 2013). While many anode-respiring bacteria prefer to oxidize simple organic compounds such as acetate, fatty acids present in more complex anode substrates can also be used to produce electricity (Kiely, Regan, & Logan, 2011).

Extensive research has been conducted with acetate as the sole electron donor in the anode. MFCs using alternative substrates to only acetate have also been established. Several analyses of the power production, COD removal rates, and microbial communities have been conducted on MFCs fed with fatty acids such as butyric, lactic, propionic, and formic acid (Freguia et al., 2010; Kiely et al., 2011). Freguia et al. (2010) looked at MFCs operating on acetic, propionic, n-butyric, i-butyric, n-valeric, i-valeric, and caproic acids fed individually as well as in a mixed feed. As expected, acetic acid was effectively removed, as well as propionic and butyric acid. In MFCs where acetate is the electron donor, *Geobacter* species are readily found, but with the presence of the above fatty acids, *Comamonas, Pseudomonas,* and *Pelobacter* species were also present. Several studies have confirmed that *G. sulfurreducens* are the most significant microorganisms in the anode when fed wastewaters, many of which are composed of sugars and fermentable end-products (Beecroft et al., 2012; Kiely et al., 2011; Parameswaran, Zhang, Torres, Rittmann, & Krajmalnik-Brown, 2010).

Mixed communities of microorganisms facilitate the breakdown of complex organic compounds present in municipal and agricultural wastewaters into simple organics which allows for both the removal of organic matter in a waste stream and electricity production (Kiely et al., 2011). Although using pure cultures in an anode may produce equal power densities as mixed communities, using mixed cultures is easier for scaling up MFCs (Nevin et al., 2008). The presence of diverse microorganisms in the anode that can simultaneously oxidize various organic substrates from complex wastewaters, make MFCs practical for wastewater treatment. Not only are organics degraded to undetectable limits, but it also reduces the internal resistance to yield high power densities (Nevin et al., 2008; Watson & Logan, 2010).

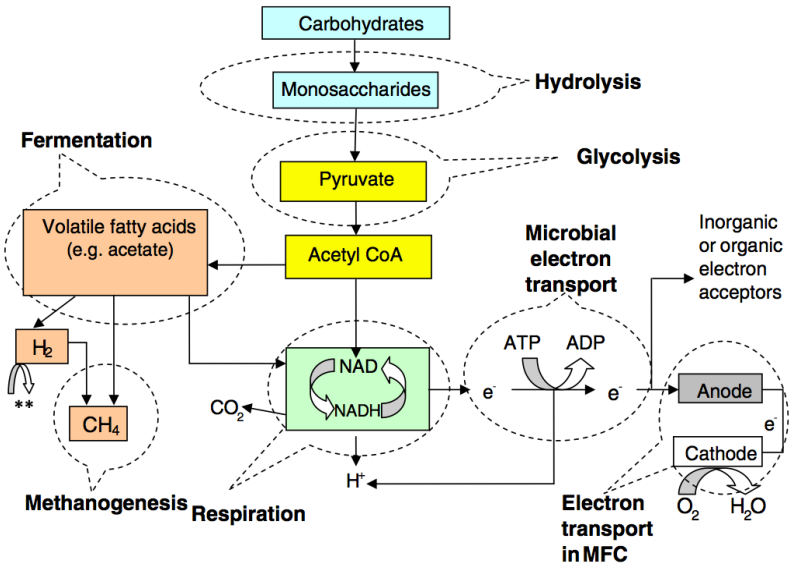

Figure 7. Diagram of substrate degradation pathways in an MFC (Velasquez-Orta et al., 2011)

Complex substrates yield low power densities due to competition between anode-respiring bacteria and bacteria that facilitate anaerobic digestion (Velasquez-Orta et al., 2011). Complex substrates can contain carbohydrates, proteins, sugars, fatty acids, and other constituents that bacteria can utilize for different metabolic pathways (Figure 7).

During hydrolysis, macromolecules are broken down into smaller molecules that can be utilized by cells for metabolism and the same microorganisms that perform hydrolysis perform fermentation to produce organic acids (Droste, 1997). Acetic, propionic, and butyric acids are found in higher concentrations than other volatile fatty acids during anaerobic digestion (Rittmann & McCarty, 2001). Fatty acids are all removed at lower rates than acetic acid, where butyric acid provides very little current production (Freguia et al., 2010)

According to Kiely et al. (2011), the rate of which complex substrates are degraded is dependent on the loading rate of the MFC. High loading rates lead to direct competition between anode-respiring bacteria and methanogens. When methanogens use the available carbon sources to produce methane, the overall coulombic efficiency of the MFC, a ratio of the amount of actual electrons transported from anode to cathode and the theoretical amount of electrons that are available if the organic substrate was completely oxidized, decreases. Since anode-respiring bacteria are slow growers compared to acetogens and methanogens, they are outcompeted for substrates and electricity production becomes limited. Therefore, there is a sensitive balance in the hierarchy of degradation of complex organic matter.

2.3 Cathode-Oxidizing Bacteria

To reduce chemical compounds, bacteria can use the cathode as an electron donor. These biologically thriving systems are called biocathodes. In MFC research, air cathodes, using oxygen as the terminal electron acceptor, have been extensively used. Although abiotic oxygen reduction with platinum-group catalysts at the cathode report higher power densities, biocathodes can be built at lower costs and are more sustainable than

using metal catalysts and mediators to complete the reduction reaction (He & Angenent, 2006; Logan, 2010). Biocathodes are versatile as they can reduce various substances. The mechanisms by which bacteria use the electrode as the electron donor has not been researched as extensively as for the anode. There are currently two proposed mechanisms: direct and indirect electron transfer. Huang, Regan, and Quan (2011) summarized electron transport mechanisms in biocathodes, concluding that microorganisms on the cathode can either excrete redox-active compounds or use exogenous mediators for indirect electron transfer while other microorganisms can transfer electrons via direct contact with the cathode surface. In oxygen biocathodes, *Acinetobacter calcoaceticus* and *S. putrefaciens* were shown to excrete redox cofactors similar to pyrroloquinoline quinone for extracellular electron transfer (Freguia, Tsujimura, & Kano, 2010).

MFCs that have microorganisms in the cathode have been able to reduce oxygen, nitrate, uranium, perchlorate, and chlorinated compounds (Butler, Clauwaert, Green, Verstraete, & Nerenberg, 2010; Franks & Nevin, 2010). Microbial communities present in a biocathodes using oxygen or nitrogen as the electron acceptor were characterized by Chen et al (2008). Community analysis revealed that Betaproteobacteria, Bacteriodetes, Proteobacteria, Chlorobi, Deltaproteobacteria, Actinobacteria, and Gammaproteobacteria were all present in the biocathode, where Bacteriodetes dominated when oxygen was the electron acceptor and nitrate-reducing bacterial species such as *Nitrosomonas* and *Azovibrio* dominated when nitrate was the electron acceptor (Chen et al., 2008). Butler et al (2010) also analyzed the community present in a denitrifying biocathode and saw

similar results, where Betaproteobacteria dominated, specifically sequences affiliated with the genera *Ferritrophicum* and *Sideroxydans*.

Biocathodes have the potential to be used for bioremediation of contaminated water sources. MFCs performing autotrophic denitrification in the cathode have already been demonstrated for the remediation of nitrate polluted groundwater (Pous, Puig, Coma, Balaguer, & Colprim, 2013). Nitrate in open water bodies can cause eutrophication and fish kills, while ingestion of nitrate by humans can cause methemoglobimenia (van Grinsven, Ward, Benjamin, & de Kok, 2006). MFCs with biocathodes are feasible for low cost sustainable systems that could be used in the developing world because they would require bacteria in both the anode and cathode as the biocatalyst rather than expensive chemical catalysts.

2.4 Electrode Material

In a MFC, the material used as the anode and cathode electrodes impact the overall performance of the MFC. Electrode materials can affect how microorganisms attach to their surfaces, the electron transfer between anode and cathode, and the rate at which the electrode is oxidized or reduced (Zhou, Chi, Luo, He, & Jin, 2011). Electrode material also affects the economic feasibility for MFC scale-up.

It is important to consider the following properties for MFC electrode materials: electrical conductivity, resistance, biocompatibility, corrosion, surface area, and strength (Zhou, 2011). Metal electrodes such as noncorrosive stainless steel mesh and gold have been used in the anode while platinum has been popular in abiotic cathodes (Xiao et al., 2012; Zhou et al., 2011). In abiotic cathodes, the electrode material often includes the addition of reduction catalysts, such as platinum, to facilitate the reduction of oxygen

(Logan, 2008). These electrodes are economically unsustainable for large scale MFCs. The most commonly used electrode material for both the anode and cathode is carbon based. For biological anodes and cathodes, graphite has been used as a low-cost and chemically inert electrode material. Graphite rods, graphite granules, graphite fiber brushes, carbon cloths, carbon paper carbon felt, and carbon nanotubes have been studied in the anode while graphite, carbon cloth, and carbon paper are common cathode electrode materials (Chen et al., 2008; Cheng et al., 2006; Liu et al., 2004; Logan, 2008; Rabaey, Clauwaert, Aelterman, & Verstraete, 2005). Graphite is a good conductor and is a more economical alternative to expensive metals.

2.5 Electroneutrality Between Electrode Compartments

The purpose of using proton exchange membranes (PEMs) in two chamber MFCs is to separate the anode and cathode electrolytes, while allowing protons to diffuse from the anode to the cathode to maintain electroneutrality between compartments (Logan, 2008). An additional benefit, PEMs prevent oxygen or other chemicals used as electron acceptors in the cathode from entering the anode. However, oxygen can diffuse through the PEMs into the anode, reducing coulombic efficiency (Butler et al., 2010). Many PEMs used in MFC applications have been adapted from conventional hydrogen fuel cell applications and are not optimized for systems operated at neutral pH, ambient temperatures, with additional cations, and in the presence of bacteria. When the PEM preferentially transports other cations over protons or the membrane become fouled with growth of bacteria, the anode compartment can become acidic, having detrimental effects on the microbial communities (Du, Xie, et al., 2011). Liu and Logan (2004) first studied a membraneless MFC using a single-chamber system with an air cathode. In the

membraneless MFC the power produced was 146 ± 8 mW/m^2, compared to an analogous MFC with a PEM which produced only 28 ± 3 mW/m^2. The increase in power output is attributed to a higher cathode potential but the Coulombic efficiency decreased from 28% to 20% due to oxygen diffusing into the cathode. In a two-chamber, membrane MFC, the anode and cathode were hydraulically partitioned in order to transport protons via advection. The flow of the electrolytes from one chamber to the other limited the diffusion of oxygen into the anode (Du, Xie, et al., 2011). Removing the PEM improved the internal resistance in the MFC because electrolyte flow is in the same direction as the proton transfer. Without PEMs, costs for scaling-up MFCs would be greatly reduced since exchange membranes are costly. The costs of two popular PEMs were estimated at $\$95/cm^2$ for Nafion 117 and $\$50/cm^2$ for SPEEK. PEMs are also susceptible to fouling over time so removing PEMs all together eliminates maintenance costs.

2.6 Pilot-scale MFCs

Only a few large-scale, field-tested MFCs have been demonstrated, and with minimal success. A pilot scale MFC consisting of 12 vertical tubular reactors, with a combined liquid volume of 1000 L was constructed in Yatala, Queensland, Australia to treat a dilute brewery wastewater. It yielded low COD removal in the anode due to biofouling in the air-cathode due to oxidation of organics in the cathode influent. (Logan, 2010). Although air-cathodes have also been shown to produce higher power densities when coupled with membrane-less MFC reactors (Hong, 2004), they become impractical for use in reactors that treat complex wastewaters. In these air-cathode systems, oxygen diffuses to the anode and biofilms accumulate on the cathodes due to incomplete removal of organics in the anode. Cusick et al. (2011) constructed a continuous flow pilot-scale

microbial electrolysis cell (MEC) of 910 L (liquid volume) to produce hydrogen gas from treating winery wastewater in Oakville, CA. After an intensive start-up period that explored pH and temperature effects on power, it produced a maximum current density of 7.4 A/m^3 and evolved 0.19 ± 0.04 L/L/day of hydrogen. MECs require external power input, making these systems nearly impossible to implement in developing countries where power sources are limited.

There are several meso-scales systems. Groups in the Netherlands and the U.S. have previously developed two different 20 L MFCs. The former group used a bipolar plate MFC stack of four cells with a total membrane surface area of 2 m^2 (Dekker, Ter Heijne, Saakes, Hamelers, & Buisman, 2009). Their MFC system was able to sustain 1.44 W/m^2 with acetate as the electron donor at the anodes and oxygen as the electron acceptor at the cathode. A platinum catalyst was used in the cathode, which makes this MFC infeasible for construction in developing areas where platinum may be too expensive and too difficult to acquire. The second MFC used 12 anodes and cathodes in two hydraulically separated anode and cathode chambers. Power production peaked at 380 mW/m^2 when feeding domestic wastewater to the anode at a loading rate of 0.66 $kg/m^3/d$ removing 80% of organic contaminants at a hydraulic retention time of 20 hours (Jiang et al., 2011). Cathodes were coated with copper and cobalt manganese oxide as the catalyst for oxygen reduction. The cathode sustained fouling due to precipitation of calcium and sodium, increasing the internal resistance from 175 Ω to 225 Ω.

To develop low cost MFC technology, it is imperative to overcome the obstacles that decrease overall power production and increase costs. Simple MFCs that could be

constructed in the most remote areas of the world have yet to be developed, but their appeal for power generation is pushing the research boundaries forward.

2.7 Research Objectives and Scope

The purpose of this research is to develop a low cost MFC that can be coupled to a composting latrine for waste treatment, energy recovery, and as an improved sanitation system for low and middle-income countries.

Design and pilot a low-cost, large-scale MFC - Only a handful of studies have investigated MFCs with volumes greater than a liter. Although power production has reached over 1 kW/m^3, many MFCs still use expensive materials such as proton exchange membranes and metal catalysts at the cathode that make them infeasible for scale up. The MFC proposed in this work uses a simple three-chamber design. Each chamber is hydraulically partitioned, eliminating the need for a proton exchange membrane. A biocathode allows for nitrate removal in the cathode and does not require expensive catalysts. A pilot MFC was constructed in the lab to validate the design for an experimental MFC latrine system implemented in Ghana. The chambers were approximately a 1:1 scale of the proposed MFC Latrine. The operational performance, including organics and nitrogen removal and power production, was monitored under different reactor conditions over a period of two years as a validation of the MFC component of the MFC Latrine in Ghana.

Investigate the breakdown of complex organic matter in the MFC anode – No MFC system has used undiluted human waste as a substrate in the anode. Many anode-

respiring bacteria are limited in their ability to breakdown complex organic matter and often rely on simple organics, e.g., acetate and glucose, for electron donors. It is likely that a hierarchy of microorganisms is responsible for degradation of complex organic matter. To better understand, the degradation of complex organic human waste in the anode, composition of organic compounds and nitrogen were monitored in the anode of the pilot MFC fed a synthetic feces and urine solution.

Deploy an MFC coupled with a composting latrine for waste treatment in Ghana – To address the growing need for adequate sanitation in Ghana, an experimental MFC Latrine was deployed. The use of construction materials that could be readily found in developing countries was prioritized as well as the use of local labor during the construction process. The MFC design created and tested in the lab was used to retrofit a newly built composting latrine in Ghana for the purpose of improving sanitation in a rural village and the performance was monitored for a year.

Assess the MFC Latrine use by local users in Ghana - Although the MFC design was validated in the laboratory, the MFC latrine's performance is constrained by the users. The usage and maintenance patterns of the MFC Latrine were observed for a year.

Chapter 3: Pilot Scale Microbial Fuel Cell for Synthetic Human Waste Treatment and Power Generation

3.1 Introduction

Microbial fuel cells (MFCs) have the potential for power generation and the ability to be completely sustainable systems. Advances in MFC research have shown that low cost systems are achievable but full-scale systems have yet to be developed to target the developing world market (Logan, 2010; Zhou et al., 2013). 2.6 billion people in low and middle income countries lack access to sanitation facilities that separate excreta from human contact, and of those, over 1 billion practice open defecation (WHO/UNICEF JMP, 2013). The liquid and solid waste that humans produce on a daily basis can potentially be used as a fuel source to power inexpensive MFCs built for the developing world. In remote areas where electricity is limited or nonexistent, MFC technology would provide a cheap and sustainable alternative.

MFCs have used domestic and agricultural wastewater as the organic substrate but these have yielded power densities much lower than MFCs using only acetate (Rabaey & Verstraete, 2005). Human waste contains a significant amount of organic and nutrient compounds, as the average person produces nearly 49.0 g of COD (chemical oxygen demand) per day and 81% to 99% of nitrogen in urine is present in the form of ammonium (Jonsson, Baky, Jeppsson, Hellstrom, & Karrman, 2005). Du et al. (2011) used diluted human wastewater and synthetic human wastewater to produce electricity in a two chamber bench scale MFC coupled to an air cathode. Using synthetic human waste

as the anode substrate, the MFC produced 15 mW/m^2. It is clear that human waste has the potential to be used as a fuel source for MFCs. Removing the PEM in MFCs that are hydraulically connected not only reduces material and maintenance costs, but it also decreases the internal resistance of the cell (Oh & Logan, 2006). Using biocathodes can also potentially reduce the cost of the system. Rather than using expensive metals and chemical catalysts that require regeneration, microorganisms can 'catalyze' cathodic reactions (Butler et al., 2010; Chen et al., 2008; Gregory et al., 2005).

In order to make use of the organic and nutrients present in human waste as well as to design a low cost MFC that can be reproduced in low-income areas, it is proposed that a separate nitrification stage that converts ammonium into nitrate be coupled to a hydraulically partitioned two-chamber MFC. The research outlined demonstrates a working pilot-scale MFC that treats synthetic human waste while simultaneously producing electricity. The three chamber system omits the most costly components of previously proposed MFC designs to create a simple process that can be coupled with a traditional composting latrine for sanitation in remote and low-income areas. The following work describes the multi-stage validation of a large-scale MFC for the removal of organic and nitrogen species in a synthetic waste stream.

3.2 Materials and Methods

The study was divided into two operational phases. Phase I was used to establish anode-respiring and cathode-oxidizing bacteria in the anode and cathode compartments and to verify sustainable electricity production under conventional MFC conditions. In Phase II, the pilot was operated under conditions that would mimic the periodic use and direct human waste to which the system would be exposed in the deployed environment.

3.2.1 Phase I MFC Reactor Set-up

For Phase I, a continuous flow two chamber MFC was set up (Figure 8). Each chamber consisted of a 56.8 L polypropylene tank with lids and connected with 1cm diameter tubing. Two baffle walls, evenly distributed within the anode and cathode chambers, were inserted into the chambers with holes lining the top of the first baffle and holes lining the bottom in the second baffle to improve flow within the chambers. The anode and cathode were both anoxic and sealed with high vacuum grease. Each tank was filled with 45.5 L of synthetic granular graphite (Graphite Sales, Chagrin Falls, OH) with a standard size raging between 2 mm and 10 mm. The total surface area of the electrode material in each of the anode and cathode chambers was estimated to be 170.2 m^2. Three graphite rods (OD: 0.625 in; L: 24 in) in each chamber were used as current collectors. A 150 Ω resistor was placed between the anode and cathode electrodes. After polarization curves were conducted, the resistance was altered to 174 kΩ.

The anode and cathode chambers were both inoculated with 4.0 L of primary wastewater obtain from the Amherst Wastewater Treatment Plant (Amherst, MA) and 1.0 L of pond water and sediments from the campus pond at the University of Massachusetts (Amherst, MA). The anode chamber was fed a 16 mM phosphate buffer with the following recipe: 1.386 mg Na_2HPO_4, 0.849 mg KH_2PO_4, 0.050 mg NH_4Cl, 0.040 mg $MgCl_2$, per liter of reverse osmosis (RO) water. For the first 91 days, the anode feed contained 616.9 mg CH_3COOK (480 mg COD) per liter. From day 92 to 158, the anode feed contained 1660 mg CH_3COOK (1080 mg COD) per liter. The cathode chamber was fed nitrate in a 16 mM phosphate buffer with the following recipe: 0.710 mg Na_2HPO_4, 1.50 mg KH_2PO_4, 0.050 mg $MgSO_4$, and 0.674 mg $NaNO_3$ per liter of RO water. One

milliliter per liter of each calcium iron solution and trace mineral solution were added to the anode and cathode feeds as a bacterial growth enrichment. The calcium iron solution consisted of 1 g $CaCl_2*2H_2O$ and 1 g of $FeSO_4*7H_2O$ per liter of RO water. The trace mineral solution contained 100 mg $ZnSO_4*7H_2O$, 30 mg $MnCl_2*4H_2O$, 300 mg H_3BO_3, 200 mg $CoCl_2*4H_2O$, 10 mg $CuCl_2*2H_2O$, 10 mg $NiCl_2*6H_2O$, 30 mg $Na_2MoO_4*2H_2O$, and 30 mg Na_2SeO_3 per liter of RO water. The Anode and cathode feeds were pumped into their respective chambers. Liquid entered the bottom of the chamber and exited through the top. Both the anode and cathode were fed at a rate of 2 mL/min. Anode effluent flowed directly into the cathode and the cathode effluent was collected in a separate waste container. Samples of the anode and cathode influent feeds and effluents were taken weekly and refrigerated. The MFC ran in Phase I for 158 days.

Initially, the anode was fed 480 mg COD/L while the cathode was fed 110 mg NO_3-N to maintain a carbon to nitrogen ratio of 4. Typical raw domestic wastewater ranges between 200 and 780 mg COD/L (Qasim, 1999), thus, the concentration used for the anode chamber was within this range. Acetate concentration was later increased to 1080 mg COD/L after 91 days of operation. The actual measured COD in the influent was significantly less than the theoretical concentration, which was attributed to degradation of acetate in the carboys where media was stored and within the anode samples before they were analyzed. Additionally, acetate was lost to alternative microbial metabolisms in the anode compartment such as sulfate-reduction. To minimize degradation within the carboys, they were cleaned with detergent and a 5% bleach solution and kept under a nitrogen atmosphere.

Additionally during Phase I, the conductivity of the anode feed solution was increased using NaCl after 74 days to ten times the original concentration (2.9 mS/cm to 30 mS/cm) to reflect the greater conductivity of undiluted human waste. During this time, a polarization curve was acquired to assess the electrochemical performance (see Section 3.2.3 for details). After 58 days of operating with a high conductivity, the high concentration of NaCl proved to be detrimental to the microbial community and began to affect power output so the conductivity was set again to 2.9 mS/cm (the original conductivity of the anode media). Conductivity and pH were measured from the acquired weekly samples.

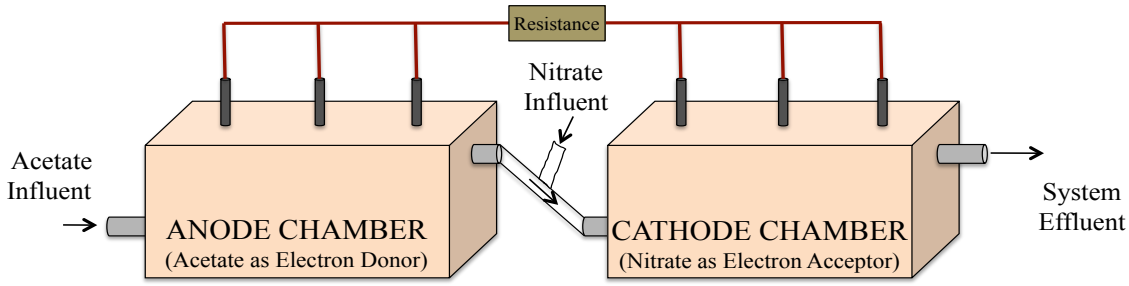

Figure 8. Phase I MFC setup with acetate and nitrate as the sole electron donor and acceptor in the anode and cathode, respectively.

3.2.2 Phase 2 Operation

In Phase II, a separate 56.8 L nitrification chamber was added to the existing MFC set-up from Phase I. Effluents of both the anode and nitrification chambers entered the cathode chamber (Figure 9). The nitrification chamber was inoculated with 6.0 L of Amherst primary wastewater and 4.0 L of campus pond water and sediments. The chamber was also up-flow and the effluent fed directly into the cathode chamber. The feeds into the anode and nitrification were fed at 2 mL/min while the flow into the cathode was 4

mL/min. To simulate the intermittent mixing of the nitrification from the introduction of new influent, an Arrow Engineering electric stirrer (1750 model; Hillside, NJ) was used to introduce air into the nitrification chamber. A ChronTrol timer (XT-4 model; San Diego, CA) was set up to mix the chamber for 1 minute every 5 minutes.

Additionally, during Phase II, the MFC feed was changed to represent the complex nature of a direct human waste stream. The anode chamber was fed a synthetic feces solution consisting of 8.0 g of starch, 2.50 g of casein, 4.34 g of KH_2PO_4, 1.09 g of Na_2HPO_4, 0.310 g of NH_4Cl, 0.130 g of KCl, and 5.0 g of $C_{18}H_{34}O_2$ (oleic acid) per liter of RO water (Du et al., 2011). The nitrification chamber was fed a modified urine solution consisting of 8.0 g of NaCl, 1.64 g of KCl, 2.63 g of K_2SO_4, 7.15 g of NH_4Cl, and 13.4 g of CH_4N_2O (urea) per liter of RO water (NASA, 1971). In addition to the sampling points in Phase I, samples from the nitrification feed and effluent were also taken weekly.

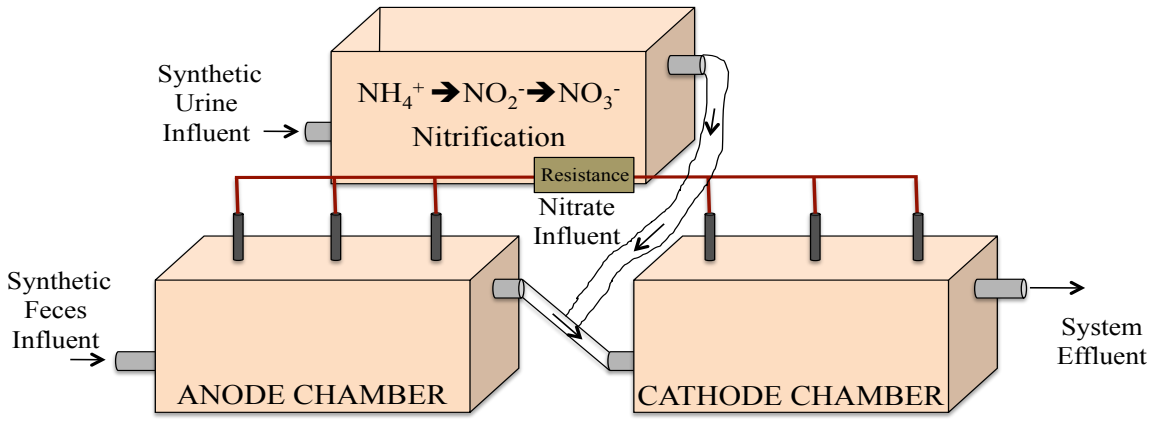

Figure 9. Phase II MFC setup with carbonaceous compounds from synthetic feces as the electron donor in the anode and converted nitrate from synthetic urine as the electron acceptor in the cathode.

3.2.3 MFC Performance Analysis

Voltage production was monitored using a Kiethley data acquisition system (model 2700, Cleveland, Ohio). Readings were collected every 10 minutes during Phase I and Phase II across the external resistance.

During Phase I, the internal resistance and peak power were characterized using polarization curves. The polarization curves where determined manually by measuring the voltage across the following external resistances (Ω): 1, 270, 510, 2.2K, 5.1K, 22K, 68K, 270K, 470K, 1M, 1.5M, and 10M. At each resistance, the voltage was allowed to stabilize for 15-20 minutes. Current was determined using Ohm's Law, $I = V/R$, where I is the current in amps (A), V is the voltage in volts (V), and R is the resistance in ohms (Ω). Power was determined using $P = I^2R$, where P is power in watts (W). Current and power densities were normalized to the cathode surface area.

A 850 Metrohm professional ion chromatograph (Riverview, FL) was used to determine concentrations of acetate, nitrite, nitrate, and ammonium. A Metrosep C 2-250 column (L: 250 mm; D: 4mm) was used for the separation of cations using a solution of 4.0 mM tartaric acid and 0.75 mM dipicolinic acid as the eluent while a Metrosep A Supp 5 column (L:250mm; D:4mm) was used for the anions. The anion column used a 3.2 mM sodium carbonate and 1.0 mM sodium bicarbonate eluent, with an acid suppressor of 200 mM nitric acid. All samples were filtered through 0.45 μm syringe filters and diluted to concentrations ranging between 0.1 and 10.0 mg/L of the respective ion in 10 mL RO solutions. Samples were analyzed for 30 minutes for anions and 15 minutes for cations.

Hach kits for low range COD were used for total COD concentrations in the anode feed and effluent during Phase II. Samples were digested for 2 hours and COD was

measured using a HACH portable spectrophotometer (DR 2800, Loveland, CO) at a wavelength of 420 nm. An Agilent gas chromatograph (GC) (7890A model, Santa Clara, CA) was used to measure the following volatile fatty acids (VFAs): acetic, propionic, isobutryic, n-butyric, isovaleric, n-valeric, isocaproic, n-caproic, and heptanoic (standard from Matreya LLC, Pleasant Gap, PA). The GC used a VFA column (DB-FFAP; L:30m; D: 0.530 mm; Film: 0.50 μm). GC samples were filtered (0.45 μm syringe filters) and acidified with 6 N of sulfuric acid before analyzing. The total nitrogen concentration during Phase II was determined using a Shimadzu total organic carbon/total nitrogen (TOC/TN) analyzer (TNM-1 model, Kyoto, Japan). TN samples from the nitrification and cathode chambers were taken within the first 70 days after initial operation of Phase II to determine to total nitrogen in the nitrification media and the cathode effluent. Samples were filtered with 0.45 μm syringe filters and acidified with 6 N of HCl before analyzing.

To demonstrate that a light could be lit with the MFC power, a 1.2 V AA rechargeable battery was completely drained and placed inside a solar landscape LED lighting torch.

3.3 Results

3.3.1 Establishing the MFC Biofilms

The purpose for the Phase I setup was to provide an opportunity for bacteria to acclimate to a typical MFC operation scheme based on previous lab-based studies (Jadhav & Ghangrekar, 2009; Rodrigo et al., 2007) and to quantify how much power and substrate removal the pilot-scale MFC design could achieve. During Phase I, the MFC was

operated under four different conditions, where acetate concentration was either 480 or 1080 mg COD/L and conductivity was 2.9 or 30 mS/cm (Table 3).

Table 3. Phase I operational conditions and results

COD in media (mg COD/L)	480	480	1080	1080
Operational Period (Days)	0-74 (74 Days)	75-91 (17 Days)	92-133 (41 Days)	134-158 (24 Days)
Coductivity (mS/cm)	2.9	30	30	2.9
Acetate Removal Rate at the Anode (mg COD/L-d)	37.8 ± 5.12	27.9 ± 5.66	118 ± 10.4	140 ± 8.40
Nitrate Removal Rate at the Cathode (mg N/L-d)	5.53 ± 1.83	16.2 ± 3.31	24.6 ± 2.65	29.5 ± 1.35
Power Density (nW/m^2)	3.40 ± 0.01	3.62 ± 0.04	1.41 ± 0.04	0.66 ± 0.02

Acetate and nitrate removal was successfully achieved under all operating conditions. In the first 91 days of operation, the MFC was able to remove an average of 72.3 ± 8.76% of acetate (as COD) at the anode, but only 36.0 ± 3.29% of nitrate as nitrogen was removed at the cathode (Figure 10). During the saline periods, acetate removal of either 480 or 1080 mg COD/L increased. When the acetate concentration was increased to 1080 mg COD/L, nitrate removal improved to 71.1 ± 3.90%, suggesting

electron delivery from the oxidization of acetate in the anode was limiting denitrification in the cathode. On an electron equivalent basis, an average of 26.3 ± 3.55 meq/L were produced at the anode during the first 91 days when acetate concentration in the influent was 480 mg COD/L but 28.9 ± 2.08 meq/L were required at the cathode to completely reduce the nitrate. Energy is also required for cell synthesis for bacteria, so there were insufficient electrons transported from anode to cathode to sustain denitrification. Nitrate removal improved even while the operational conditions changed, designating potentially favorable conditions at the cathode.

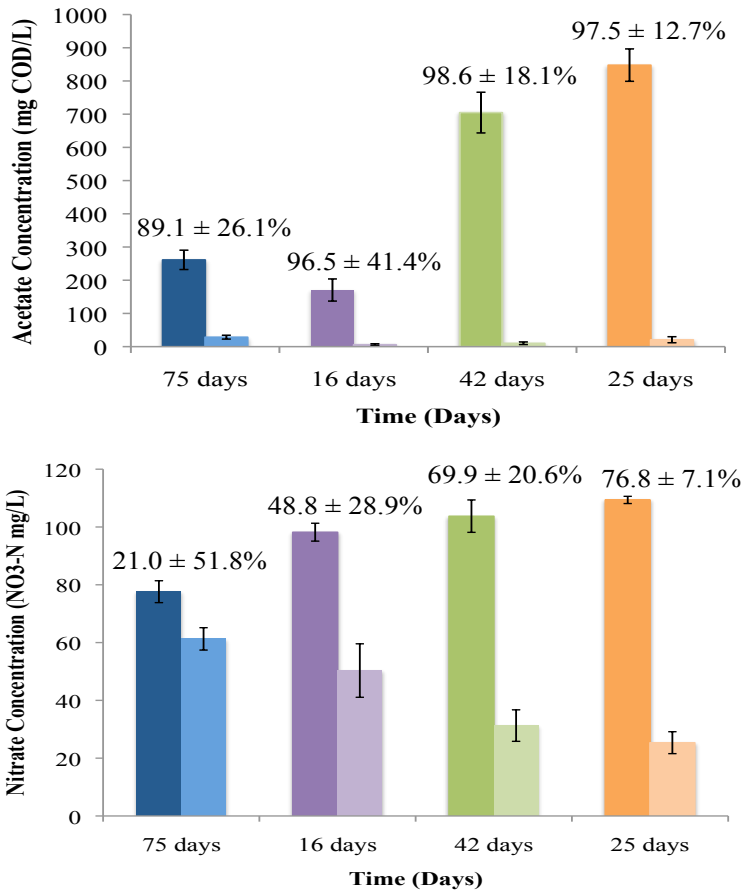

Figure 10. Acetate and nitrate concentrations during Phase I. Dark shade colors represent the influent concentrations, while the lighter shades represent the effluent. Organic loading limited the nitrate reduction observed in the cathode during the first 91 days of operation.

Power production during the first 74 days was low, averaging at 3.40 ± 0.01 nW/m^2. The surface area of each electrode was large, 170 m^2, and the microorganisms may not have achieved complete surface coverage, leading to a smaller representation of power production. To reflect the conductivity of direct human wastewater that the proposed MFC system would treat, the conductivity was increased to 30 mS/cm. Polarization curves highlight the effect of conductivity on potential power production (Figure 11). Power output was observed to increase by a factor of 2.5 with a 10-fold increase in conductivity. The internal resistance of the MFC was 174,000 Ω before the addition of NaCl and decreased to 22,000 Ω after salt was added.

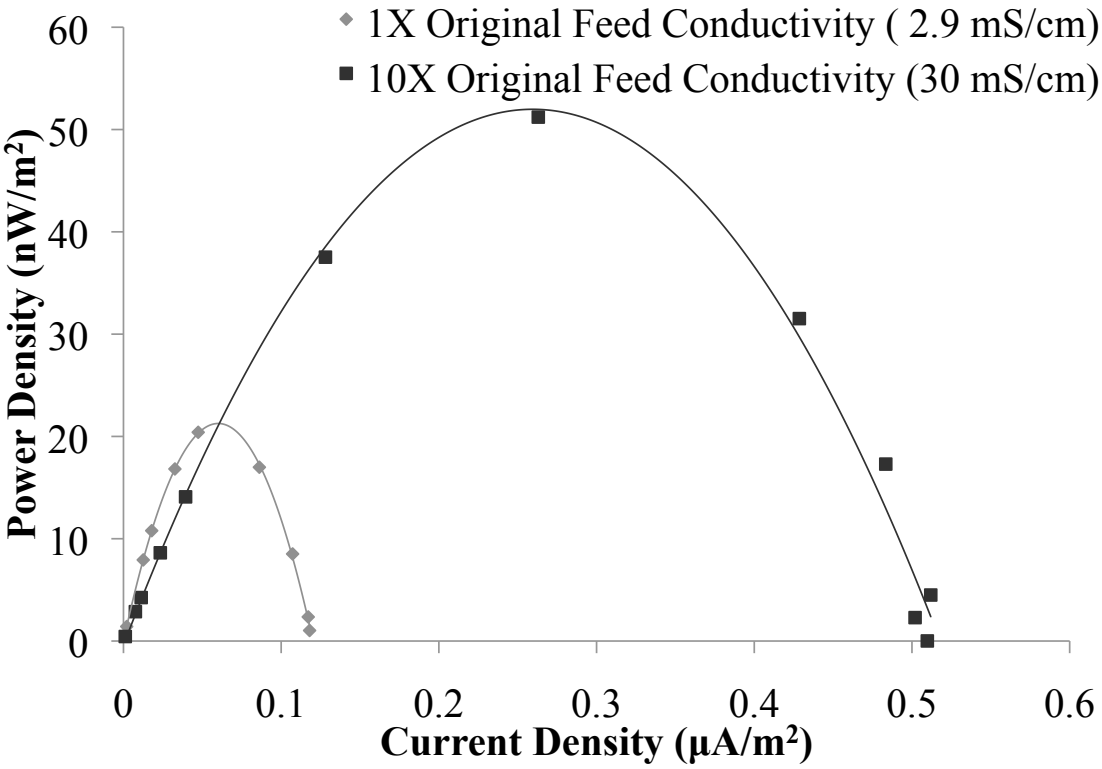

Figure 11. Polarization and power density curves for two different anode feed conductivities (O for voltage at 2.9 mS/cm and □ for voltage at 30 mS/cm)

The sustained operation of the MFC produced different results. Power production initially increased slightly and this increase was sustained for several days. However, the long-term effect of increased salinity was detrimental to power production (Figure 12). Power production returned to original levels after the conductivity was returned to 2.9 mS/cm, suggesting that the salinity may have a negative effect on the microbial communities. Interestingly, nitrate reduction increased over this period of time.

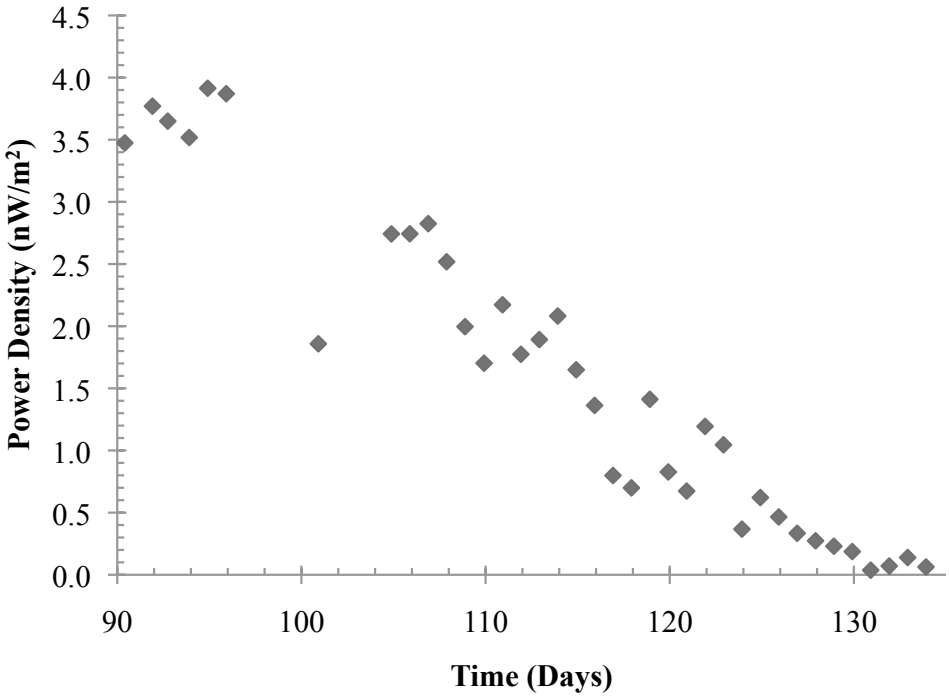

Figure 12. Power densities during high saline conditions

3.3.2 Piloting the Removal of Synthetic Human Waste

During Phase II, the nitrification chamber was set up in parallel with the anode, as shown in Figure 9, and synthetic feces and urine were fed into the anode and nitrification chambers, respectively, in order to simulate how a large scale MFC using human waste would perform.

Total nitrogen removal was observed between the nitrification stage and the cathode effluent within the first 70 days of Phase II. The total nitrogen in the influent to the nitrification chamber was on average 8560 ± 110 mg N/L. 6000 mg N/L entered in the from of urea while the rest was ammonium. The total nitrogen removed within the nitrification and the cathode chamber was 68.4 ± 2.81%, providing a system effluent with 2690 ± 55.2 mg N/L during the first 70 days of operation. The ammonium concentration entering and exiting were on average 2546 ± 70 mg N/L and 2833 ±172 mg N/L throughout Phase II, respectively.

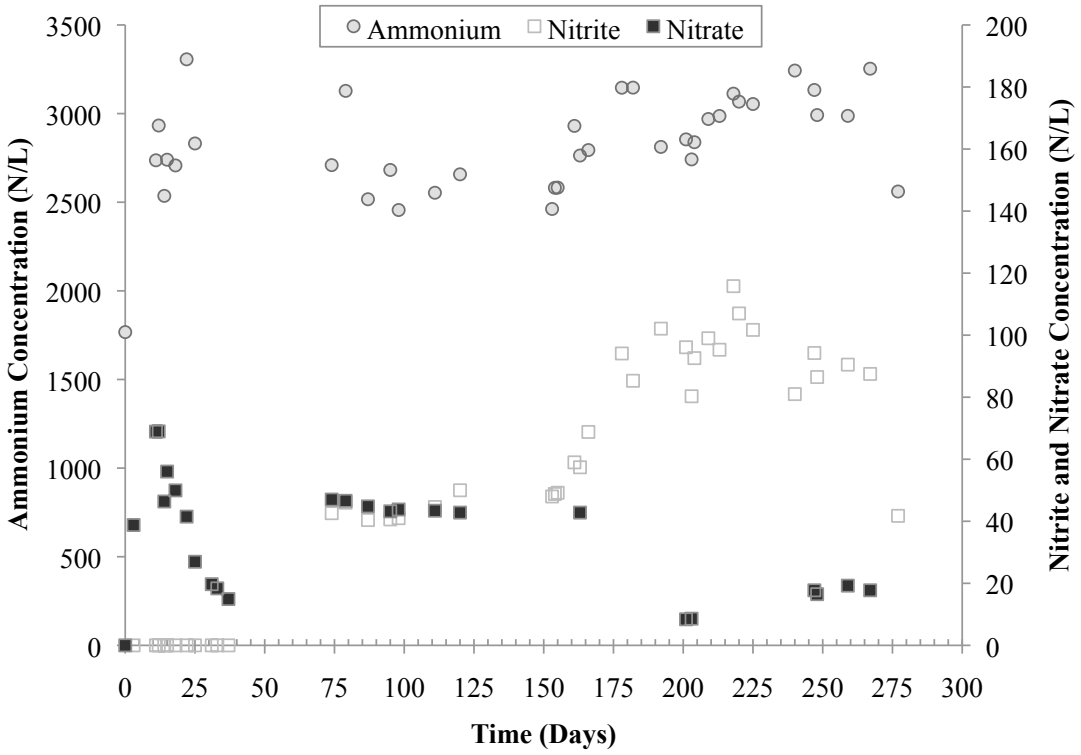

Figure 13. Concentration of nitrogen species in the nitrification chamber during Phase II

Figure 13 highlights the nitrogen species present in the nitrification chamber during Phase II. Ammonium oxidation was observed in the nitrification chamber, where nitrite and nitrate were both observed as partial and total nitrification products, respectively. Ammonia oxidation was expected to occur within the nitrification process,

where ammonium and nitrate concentrations would be low and intermediates such as nitrite would ideally not occur. It was evident that partial nitrification was occurring, where higher nitrite concentrations into the cathode influent were observed than nitrate concentrations.

Nitrogen species were also monitored in the cathode chamber (Figure 14). In the cathode, nitrate reduction occurred during the beginning and end periods of Phase II. Between days 75 and 170, nitrate concentrations in and out of the cathode remained the same, while nitrite removal was observed. Nitrite removal continued until the end of Phase II.

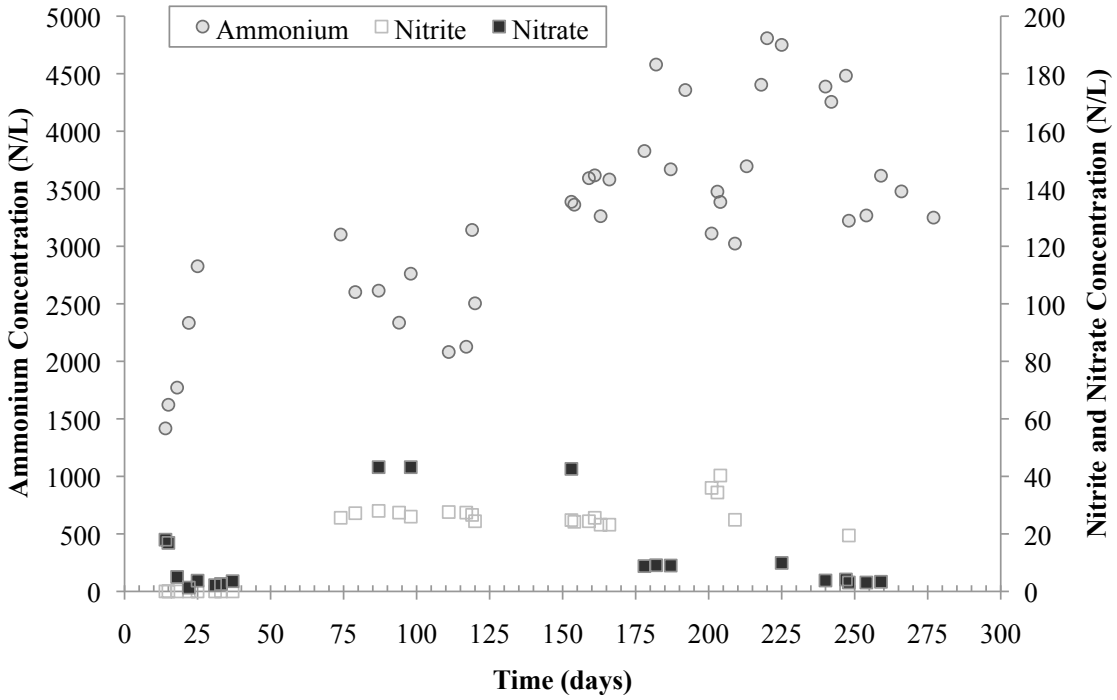

Figure 14. Concentration of nitrogen species in the cathode chamber during Phase II

The synthetic feces media entering the anode chamber was composed of significant quantities of carbon sources: starch, casein, and oleic acid. The COD concentration during Phase II was a magnitude higher than the observed average of 760 ±

44.7 mg COD/L seen at the end of Phase I. The removal rate of COD at the anode was 1110 ± 290 mg COD/L-d, where nearly 92% of the COD was removed. Along with COD, the transformation of short chain VFAs were also observed at the anode. The three major VFAs found at the anode were acetic acid, propionic acid, and n-butryric acid (Figure 15). It was expected to see acetic acid at the anode influent since the anode media contained small amounts of mustard to act as an emulsifier between the anode liquid and the oleic acid (an oil). The occurrence of propionic and n-butryric at the anode influent suggest biodegradation of the carbon sources in the media bottle before even entering the anode chamber. All other VFAs had observed concentrations below 5.50 mg of VFA/L and no significant changes between the anode influent and anode effluent were observed.

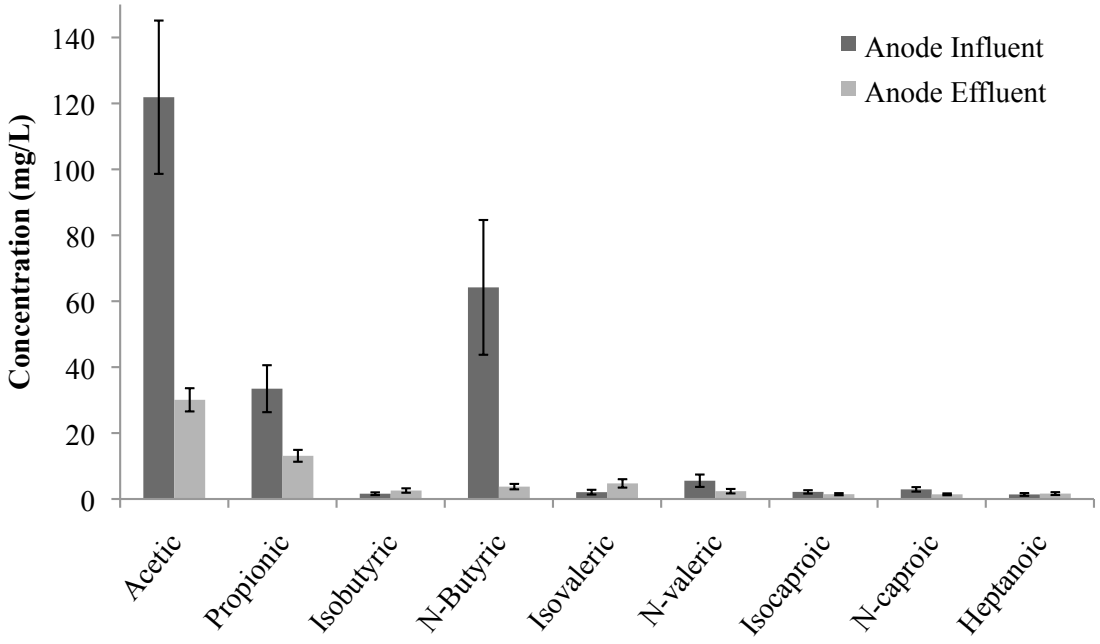

Figure 15. Concentration of volatile fatty acids at the influent and effluent of the anode chamber during Phase II

Although the COD content in the anode was significantly greater than in Phase I, the power output of the MFC treating synthetic feces and synthetic urine decreased to 1.22 ± 0.03 nW/m^2. Nevertheless, power production was sustained in the MFC throughout the experiment. Although the power density was small, the power generated is usable to power a small LED light. At the end of Phase I, the MFC was allowed to charge the battery overnight (estimated 14 hours) and lit the light for 30 minutes (Figure 16). Further studies were not completed to see how long it took to charge the battery with the MFC.

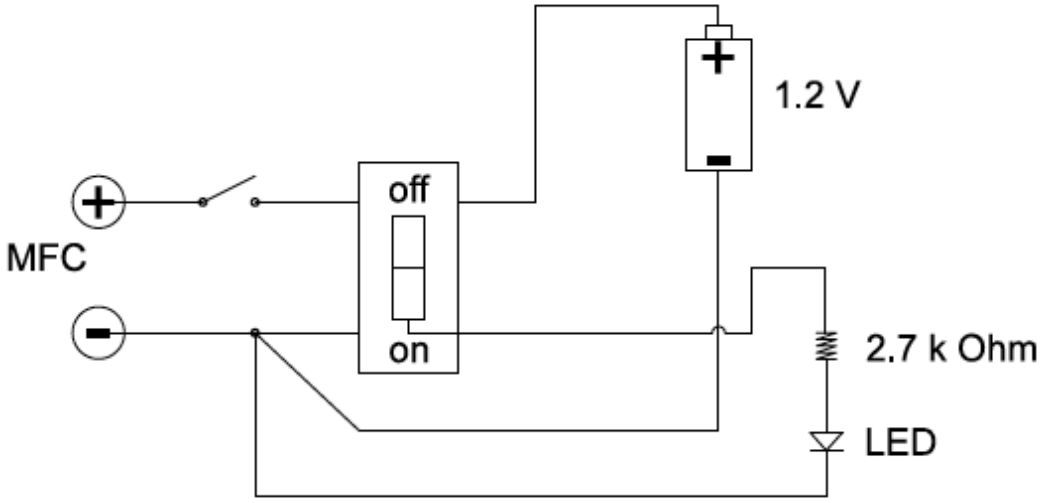

Figure 16. Circuit schematic of the LED light powered by the MFC

3.4 Discussion

3.4.1 Power Performance

During the Phase I polarization study, the maximum power (Figure 11) reached 10.3 nW/m^2 when the conductivity of the anode media was increased to 30 mS/cm. The effect of ionic strength on MFC performance has been researched by Lui et al. (2005), and has shown that power production is affected by conductivity. In the Liu et al. MFC,

an 85% power increase was observed with the addition of 300 mM of NaCl in the anode chamber. Similarly, the peak power for the MFC in this project increased by 245% during the analysis of the polarization curve, but it was not sustained. Although saline conditions can be harmful to a multitude of anode respiring bacteria, recently a species of anaerobic halophilic anode respiring bacterium, *Geoalkalibater subterraneus*, has conducted electricity through direct electron transfer (Carmona-Martinez, Pierra, Trably, Bernet, 2013). This species was obtained from sediments in a salt plant. It seems unlikely that such saline environments would be replicated within the primary clarifiers and campus pond from which the MFC inoculum was obtained. On the other hand, anaerobic halophilic fermentative bacteria also exist (Kivisto, 2010). If these halophilic fermentative organisms began to thrive and dominate during the saline conditions, it would explain why power decreased during the saline period but acetate removal was still maintained. In Phase II, when the MFC began to treat synthetic human waste, the anode and nitrification media conductivities were approximately 4.90 and 17.0 mS/cm, respectively, so the anode was not maintained under higher concentrations of salts. Although conductivity can change the power performance of the MFC, it is not the only factor that affects the energy conversion.

In both phases, power production may have also been inhibited by limitations at the cathode. Since the anode and cathode were hydraulically connected, the remainder of organic compounds not oxidized at the anode traveled to the cathode. These organic compounds served as electron donors at the cathode, creating competition between heterotrophic denitrifiers and the autotrophic denitrifiers that use the cathode as an electron donor. Similar behaviors have been observed with continuous flow MFCs (Du,

Xie, et al., 2011), where residual organic compounds unable to be removed from the influent wastewater at the anode were oxidized at the cathode. Similar to the MFC in this study, Du et al. designed a membraneless, continuous flow, two chamber reactor that used acetate as the electron donor in the anode and oxygen as the electron acceptor in the cathode.

The energy conversion by the MFC was not optimized, as shown by the low power production during both phases. However, the concentration of organic compounds removed at the anode was significant, through both Phase I and Phase II, not falling below an average of 70% removal. Under all operation conditions, removal of organics in the anode was high, reaching up to 92.4% removal but power production was very low. Power outputs were lowest during Phase II operation where COD concentrations were high.

3.4.2 Nitrogen Transformation

For nitrogen removal, the Phase I condition with 1080 mg COD/L and 2.9 mS/cm improved nitrate reduction at the cathode than when acetate concentration was 480 mg COD/L. In Phase II, conversion of ammonium to nitrate at the nitrification chamber was observed early but decreased over time. Transformation of ammonium to nitrite was also observed. Accumulation of ammonium in the cathode chamber as well as low concentrations of nitrite would suggest partial nitrification at the nitrification stage. Partial nitrification limits the amount of electron acceptor available at the cathode, affecting the overall performance of the MFC. Likewise, Removal of total nitrogen in the cathode chamber reached 65%, but much of that removal was unaccounted for through the nitrite and nitrate measurements. Precipitates along the top of the nitrification

chamber and within the tubing were observed and may suggest the occurrence of urea hydrolysis. Furthermore, nitrite was observed in high concentrations in the nitrification chamber and was removed in the cathode. One potential hypothesis is that anaerobic ammonium oxidation (anammox) is occurring within the cathode chamber. Using nitrite as the electron acceptor, ammonium is oxidized to nitrogen gas under anoxic conditions (Hu, 2011). Community analysis of the biofilms that exist within the cathode will yield insight into what bacteria exist and which organisms are driving the transformation of nitrogen. Nevertheless, transformation of nitrogen through various pathways was achieved.

As a nutrient treatment method for sanitation systems that use undiluted human waste, the MFC reactor provides nitrogen removal while producing power. The MFC can be classified under ecological sanitation facilities, which are improved sanitation systems that recover and recycle nutrients and organic matter from human waste (Esrey et al., 2001). While other ecological sanitation systems may divert urine and recover the ammonia as an agricultural fertilizer, the MFC latrine can provide energy in the form of electricity in areas where electricity is more important than fertilizer (Vinnerås, Jonsson, Solomon, & Stinzting, 2004).

3.4.3 Organic Matter Removal

In Phase II, organic substrates were complex compared to the COD supplied as acetate in Phase I. Anode-respiring bacteria are known to use acetate as an electron donor, making COD in Phase I completely accessible to bacteria respiring at the electrode. In Phase II, when more complex organics were introduced to the anode, it is hypothesized that the community of anode-respiring bacteria in the anode chamber may have been

outcompeted by bacteria that can oxidize the complex organic electron donors. Fermentors, acetogens, and methanogens are a group of microorganisms that facilitate anaerobic digestion of organics. Since organic compounds serve as the electron donors and acceptors during the fermentation process (Rittmann & McCarty, 2001), this could explain why the COD is effectively removed at the anode while the energy conversion is nearly nonexistent.

Acid- forming bacteria produce acetic acid as well as butyric and propionic acid. According to Rittman and McCarty, acetic, propionic, and butyric acid are found in higher concentrations during anaerobic digestion. In the anode chamber of the MFC, these three compounds were found in abundance, in relation to the other measured low molecular weight acids (Figure 15). Likewise, since anode-respiring bacteria are slow growers compared to acetogens and methanogens, over time, the slow growing bacteria will be out competed (Esteve-Núñez, Rothermich, Sharma, & Lovley, 2005; Lee, Parameswaran, Kato-Marcus, Torres, & Rittmann, 2008). The pH in the anode remained steady between 6.0 and 8.0, averaging 7.6 ± 0.06 during Phase I. In Phase II, pH ranged between 5.5 and 7.5 (average 6.5 ± 0.11). The shift in average anode pH suggests favorable conditions for acid forming bacteria growth, since their optimal pH is between 5.0 and 6.0. Both methanogens and anode-respiring bacteria prefer a neutral pH, while some methanogens can exist in more extreme pH environments (Ferry, 1994). Methane production was not analytically measured for this MFC but would aid in accounting for the conversion of acetic acid to methane.

3.4.4 Design Challenges

As is common with large reactors (Cusick et al., 2011), maintenance became a critical component of performance. Observations in the operation and maintenance of the pilot system will influence the design and implementation system in developing countries. The synthetic feces media was thicker than typical liquid media used for lab-based MFC research and clogging at the anode influent was observed. Bacterial growth was observed in the tubing connections at the anode influent and effluent. Using larger tubing size would alleviate the frequency of maintenance. Moreover, algal growth was also observed within the clear tubing and inside the anode and cathode chambers. The tubing was cleaned periodically to remove algae growth and connections were covered with aluminum foil. Algae growing at the top of the anode and cathode chambers were manually removed twice, once during Phase I operation and once at the end of Phase II. Preventing algae from growing with the chambers could increase the amount of ammonium and nitrate available for use by other microorganisms.

In practicality, the total surface area estimated might yield misrepresentation of the actual surface area that is being utilized by bacteria. As biofilms of microorganisms develop around the granules and oxidize the organic compounds in the anode, electrons must be transported conductively from the graphite granule to the graphite rod. Rectangular in shape, the MFC is designed as a plug flow reactor where baffle walls were added to reduce dead zones. These dead zones reduce the available reactor volume and ultimately the surface area available for microbial growth.

For real world implementation of this MFC design, modifications are required to improve nutrient removal and power production. For the system to be successful in

developing countries, minimal maintenance is required. Improving utilization of the electrode surface area by anode respiring bacteria and minimizing the flow of organics into the cathode would allow for further development of an in-situ design to treat human waste from latrines.

3.5 Conclusion

A large scale MFC treating synthetic human waste was developed for power generation. For this experiment, organic matter removal was effectively achieved at the anode when COD was introduced as acetate or synthetic waste. Although nitrogen removal was over 70% during Phase I, nitrogen removal during phase II was limited due to partial nitrification in the nitrification chamber when treating synthetic urine. Analysis of the microbial communities within the cathode would yield insight into the potential pathways in which nitrogen is being transformed. Power production was low, achieving a maximum average of 3.62 ± 0.04 nW/m^2 during phase I when the anode was treating for acetate and the conductivity was altered. Conductivity was shown to improve power production but it was not sustained. Long-term exposure to NaCl became unfavorable for power production. The large scale MFC developed for this study is the first to utilize a denitrifying biocathode and a separate nitrification stage in a three chamber system to treat synthetic human waste. The design carried out has the potential for real world applications in the developing world, with modification that can support the use of anode-respiring bacteria as the oxidizing catalyst in the anode.

Chapter 4: Case Study: Deployment of the Microbial Fuel Cell Latrine

4.1 Introduction

Less than half of the population in sub-Sahara Africa has access to improved sanitation facilities (WHO/UNICEF JMP, 2013) There is limited access to sanitation facilities in urban environments in the developing world, and that access diminishes as looking towards rural environments. In Ghana, a prospering country within the African nations, 33% of rural communities practice open defecation and that value has been increasing over the past 20 years (WHO/UNICEF JMP, 2012). Despite relative economic stability, 15,000 children under the age of five die from diarrheal diseases each year due to lack of sanitation infrastructure.

While centralized facilities are viable in metropolitan hubs like Accra and Kumasi, many districts in the northern region live in extreme poverty. In the northern regions, 63% of the population lack adequate food and water (Debrah, 2013). The most prevalent limitation in this area is food security, access to electricity, and access to clean sources of water and sanitation. For many women, it is particularly challenging to find a private place to relieve themselves so they wait until the evening. Several women have been bitten by snakes (Antwi, 2013). Access to sanitation facilities combined with improved access to quality drinking water can reduce water-borne diseases (Esrey & Habicht, 1986; Fewtrell et al., 2005). Rural areas are unable to support water distribution systems and sewer infrastructure. Communities may also be financially unable to make investments in

sanitation facilities (Cairncross, 2003). Low-cost, decentralized sanitation systems paired with community engagements is a successful way to expand sanitation coverage (Montgomery & Elimelech, 2007).

Decentralized sanitation facilities often used in rural areas of developing countries include pit latrines, composting latrines, pour-flush latrines, and flush toilets (Mihelcic et al., 2009). These systems view human excreta as a waste product rather than as a reusable resource. Ecological sanitation (ecosan) toilets are based on the reuse and recycle of nutrients found in excreta and can provide continual agricultural benefits as well as minimizing water pollution (Christine Werner, Schlick, & Mang, 2003). Where water is a limitation, urine diverting dry composting latrines not only provide a safe contained environment for excrement disposal and odor control, but also provide human waste compost and fertilizer as a soil amendment.

To address the sanitation needs in Ghana, a novel microbial fuel cell (MFC) design that can retrofit composting latrines to treat human excreta and provide compost, electricity and treated water was developed. MFCs are a novel technology for energy production because they produce electricity directly from the removal of organics and nitrogen in wastewater. Previous research has shown that a two-chamber MFC can simultaneously remove carbon in the anode and nitrogen in the cathode using a separate aerobic nitrification stage (Virdis, Rabaey, Yuan, & Keller, 2008). Bench-scale systems have yielded over 1 kW/m^3 using acetate as a fuel source and diffused oxygen as the terminal electron acceptor in the cathode (Fan, Hu, & Liu, 2007; Nevin et al., 2008). Although these systems can produce a significant amount of power, they are very expensive and impractical for the developing world. The focus of current research on

MFCs has been on bench scale systems and their potential for high power production. Many bench-scale systems use abiotic, platinum-catalyzed, oxygen-reducing cathodes similar to conventional hydrogen fuel cells. Additionally, they require expensive proton exchange membranes (PEM) to partition the anode and cathode chambers. Very little is known about the performance of large-scale MFC systems and their ability to directly treat human waste. While only a few large-scale reactors have been produced (Logan, 2010), to the best of our knowledge, no large-scale MFC has used undiluted human waste directly as the substrate in the anode and cathode as well as deployed an in-situ pilot system in the developing world.

The construction of a composting latrine and microbial fuel cell combination that will treat human waste, compost solids and produce electricity, called the MFC Latrine, was proposed for this study. The MFC Latrine attempts to eliminate the high cost elements of bench-scale designs, reducing the cost to a practical level for use in developing areas. In the proposed gravity driven, step feed MFC, the anode and the cathode are hydraulically partitioned, eliminating the need for a PEM. Low-cost graphite granules serve as electrodes and the reduction of nitrate at the cathode is facilitated by microorganisms, not expensive catalysts. Additionally, the microbial communities within this MFC are known to have low growth yield, generating little biomass and reducing maintenance requirements.

The goal is to develop a technology that will provide a safe method for sanitation as well as providing two incentives for sanitation development: compost and electricity. These incentives will encourage adaptation and proliferation of the MFC Latrine as its products can be monetized for economic benefit. For example, parties deploying MFC

Latrines could sell compost or access to electricity to fund sustained operation and maintenance. As such, the MFC Latrine may be advantageous and beneficial in areas where decentralized sanitation is developed through sanitation-as-a-business model (SAB), which is an emerging approach being undertaken by several aid-organizations (Breslin & Bramley, 2010)

This work presents a case study of the first MFC Latrine that was deployed in Ghana. The primary goals of this project are to 1) demonstrate the first field-operated full scale MFC producing electricity, 2) evaluate the MFC Latrine performance in terms of water quality and power and 2) assess the local user interface with this new technology.

4.2 Methods

4.2.1 Support

The team made two trips to Ghana for this project in May 2012 and May 2013. Deployment of the MFC Latrine was executed with significant support from individuals and organizations in Ghana. Paramount Chief, Nana Bonsu, served as our primary adviser and assisted with site selection, materials acquisition and hiring local labor. Agona Nyakrom Secondary Technical School (NYASTEC) was also an active partner and helped with identification of the optimal site on its campus as well as operation and maintenance of the MFC Latrine. After our team left Ghana, data collection was performed by Mary Kay and Charlie Jackson of Pure Home Water on a periodic basis following construction.

4.2.2 Site Selection

NYASTEC was selected as the general study site for several reasons. First, NYASTEC and the surrounding area had an established need for additional sanitation facilities (Ghana News Agency, 2011). However, the school had existing sanitation facilities. In this environment, there was a balance between need and existing sanitation resources. The MFC Latrine had a supplemental role in the local sanitation capacity without being the primary source of sanitation. This dynamic was desirable for an experimental pilot system. Secondly, as a technical high school, NYASTEC had a community (e.g., science teachers and students) who were more likely to be invested in the experimental pilot study. It was anticipated that the MFC Latrine could serve as a "living laboratory", and enhance educational opportunities in the school by exposing the students to the application of scientific principles with the MFC Latrine.

NYASTEC is located in the Central region of Ghana, in the Agona West Municipal District at the village of Nyakrom (5.62^{o} N, 0.78^{o} W), with an estimated population of 23,000. The climate in the area is tropical with typical daily high temperatures near 32 ^{o}C for the majority of the year. Rainfall varies considerably during the year, peaking in June where the average monthly rainfall is approximately 22 cm. This wet-season rainfall required design considerations in the MFC Latrine. Slope stability and drainage measures were included in the site layout, and rain gutters were included in the roof system for the latrine.

Though the site sub-surface conditions were not quantified, it was clear that excavated soils were clay-like. This was advantageous to excavation due to the inherent stability in the clay soil. However, this also meant that infiltrations rates were relatively

slow. Therefore, the effluent from the MFC Latrine was fed to an infiltration area filled with gravel. The infiltration area was located near the root system of a large tree and other vegetation to encourage water uptake.

The MFC Latrine was located in a highly traveled, central location on campus, near several academic buildings. Therefore, the anticipated user group was any student or faculty member that had the need for sanitation in that area of campus. The boarding school has 1500 students, 550 of which are female whose ages ranged between 13 and 19 years. Additional sanitation facilities at the school include 36 flush toilets available in the dormitories, and 12 are dry pit latrines (Garbrah, 2013). The MFC Latrine was sited next to a recently constructed western-style toilet facility, equipped with 12 flush toilets, and sinks for hand washing. At the time of deployment, however, the toilet facility was not connected to piped water or electricity and was not in use. Students were observed to use a nearby unimproved pit latrine for sanitation.

4.2.3 Basis for Design

The MFC Latrine design was based on a urine diverting composting latrine, similar to others deployed in developing areas of sub-Saharan Africa (Morgan, 2007; Breslin, 2002; Jackson & Knapp, 2005). The latrine superstructure was primarily constructed of concrete block and mortar and has been well documented and field proven by others such as Ludwig et al. (1988), Tawney (2006), and Mihelcic et al. (2009). The MFC Latrine has the addition of the MFC components (Figure 17). The design partially diverts urine from the composting chamber where solids are composted aerobically and the remaining liquid is transported by gravity through the MFC. Electrical current is

generated through the biological treatment of organics and nitrogen in the waste stream, which is filtered to the subsurface.

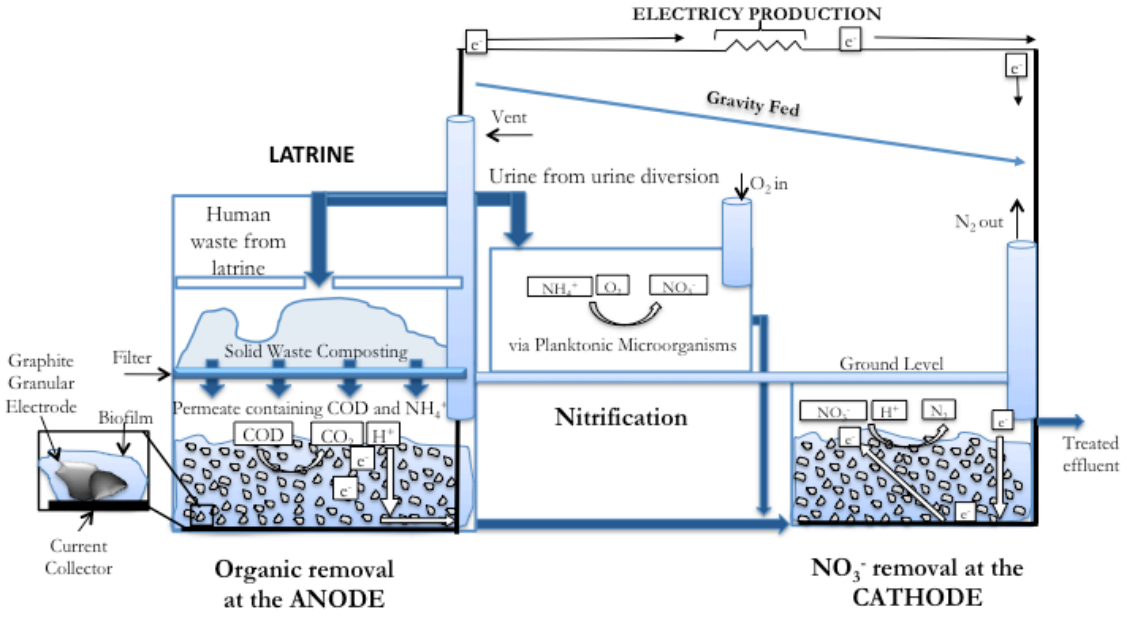

Figure 17. Design of the MFC Latrine in Agona, Nyakrom, Ghana

The MFC was designed for minimal material use and simple construction techniques to account for potentially limited resources in the study area. All materials for the MFC Latrine were acquired locally, with the exception of granular graphite electrodes that were shipped from the United States to Ghana.

4.2.4 MFC Set-up

The MFC consists of three chambers: the anode chamber, the cathode chamber, and the nitrification chamber. Communities of microorganisms in the anode oxidize dissolved organic matter from the liquid effluent that permeates through the composting chamber. Simultaneously, urine is diverted by the urinal into the nitrification chamber, where communities of ammonia-oxidizing bacteria oxidize ammonium (NH_4^+) into nitrate (NO_3^-). Effluent streams from both the anode and nitrification chamber enter the

cathode, where nitrate is reduced to nitrogen gas by another community of microorganisms. The anode and the cathode are anoxic and below grade, while the nitrification chamber is aerobic and at grade.

A 208-liter (55 Gal) drum was used for the nitrification chamber. The tank was laid on its side and two screened vents were added to the top of the drum to allow air to enter, creating an aerobic environment needed for nitrification. The chamber was oversized so that the anticipated liquid volume was approximately 25% of the capacity. This was done in order to increase the surface area of the liquid-air interface and promote oxygen transfer. The effluent port was placed roughly ⅓ of the drum diameter above the ground and the influent port was slightly higher to develop an appropriate hydraulic grade line.

Graphite granules of a relatively large size (diameter > 5 mm) filled approximately 66% of both the anode and cathode chambers. The estimated available liquid volume for the anode and cathode chamber was 40 Liters and the estimated accessible electrode surface area was 25.2 m^2. The electrically conductive graphite granules along with graphite rods act as a surface for biofilm formation and the wiring allowed for electron transfer between the anode and cathode. Both the anode and cathode were inoculated with several liters of water from a nearby well that was known to be non-potable and likely biologically impacted. In addition, dog food was added as a source of nutrients to promote bacterial growth.

A circuit was constructed to deliver electrical power from the MFC to power a light emitting diode (LED)-based light. The circuit contained a 1.2 V AA rechargeable battery that was charged from the MFC. This managed the fluctuations in power

produced from the MFC. The goal was to charge the battery during daylight hours and power the light located inside the latrine during darkness. When the circuit is in the off position the battery will charge and the LED will remain off. When the circuit is in the on position current will flow through the LED from the charged battery.

4.2.5 MFC Operation and Data Collection

Our Pure Home Water partners visited the site monthly over the first 6 months of operation. Data was collected for the soluble constituents in the anode, cathode and nitrification chambers including conductivity, pH, ammonium, and nitrate. Voltage, resistance and current were monitored across the anode and cathode. A Vernier LabQuest 2 (Beaverton, OR) and Vernier probes for the ammonium, nitrate, pH and conductivity were used. A RadioShack multimeter was used to measure voltage, current, and resistance. Power is reported in absolute terms (P=VI). In MFC research, power densities are often reported as watts/volume of liquid waste or chamber volume or watts/surface area of electrode material. Since the volume of liquid in the electrode chambers was variable and not regularly measured and likewise the accessible surface area varied, the power was provided in absolute terms.

4.2.6 Education and Maintenance Plan

To accomplish a successful deployment of the MFC Latrine, our team taught a seminar to faculty and students to show how the MFC Latrine worked and what maintenance needed to be performed. Copies of the presentation were shared with the science teachers for future reference. We also appointed several science teachers and students to take on leadership of the latrine. Their main role was to monitor the maintenance of the latrine and replace toilet paper, woodchips, and charcoal ash when

needed. A construction and maintenance manual was also provided to the school headmaster.

4.3 Results

4.3.1 Deployment of the MFC Latrine

In May 2012, the first MFC Latrine was deployed at NYASTEC (Figure 18). Construction of the system took two and a half weeks by a team of local masons and carpenters assembled by Nana Bansu. The system was put to immediate use after completion. The MFC Latrine was monitored for a period of 1 year and over the course of that year, the latrine was used regularly. After 1 year, the first composting chamber

Figure 18. The MFC Latrine Installation at NYASTEC in Agona Nyakrom, Ghana. MFC Components (top); finished MFC Latrine (bottom left); latrine interior (bottom center and bottom right)

was closed and the second opened.

4.3.2 MFC Latrine Performance.

Power production in the system began low and decreased when NYASTEC recessed for summer break, demonstrating that power production was correlated with use (Figure 19A). While school was in session, power production was consistent with our pilot study results, where the MFC in Ghana produced an average 0.17 μW before school recess and our pilot system averaged 0.21 +/- 5.4 μW. During the follow up trip in May 2013, power was observed at 6.75 μW. Total resistance decreased over time, to a minimum of 0.5 kΩ by the end of the year-long study (Figure 19B).

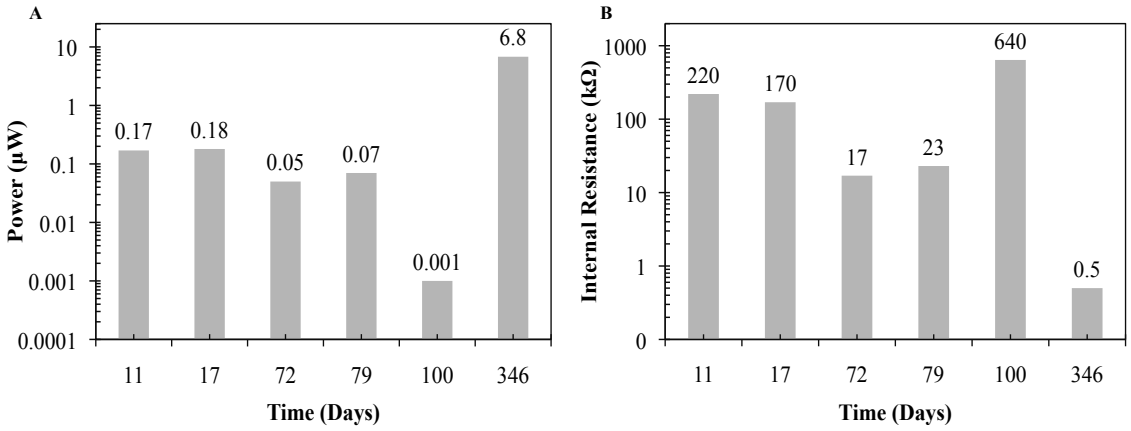

Figure 19. Power performance of the Ghana MFC Latrine. Power produced by the MFC Latrine (A); Measured internal resistance (B)

The internal resistance within the components of the MFC (i.e. anode, cathode, and electrolyte) limits power production (Logan & Regan, 2006). The ionic strength and pH of the electrolytes within the MFC affect the overall power production (Fan, Sharbrough, & Liu, 2008). pH levels at the beginning of the study began near 5.0 and increased to 7.0 in both the anode and cathode. This suggests that the internal resistance may have decreased over time, decreasing the overall resistance of the fuel cell.

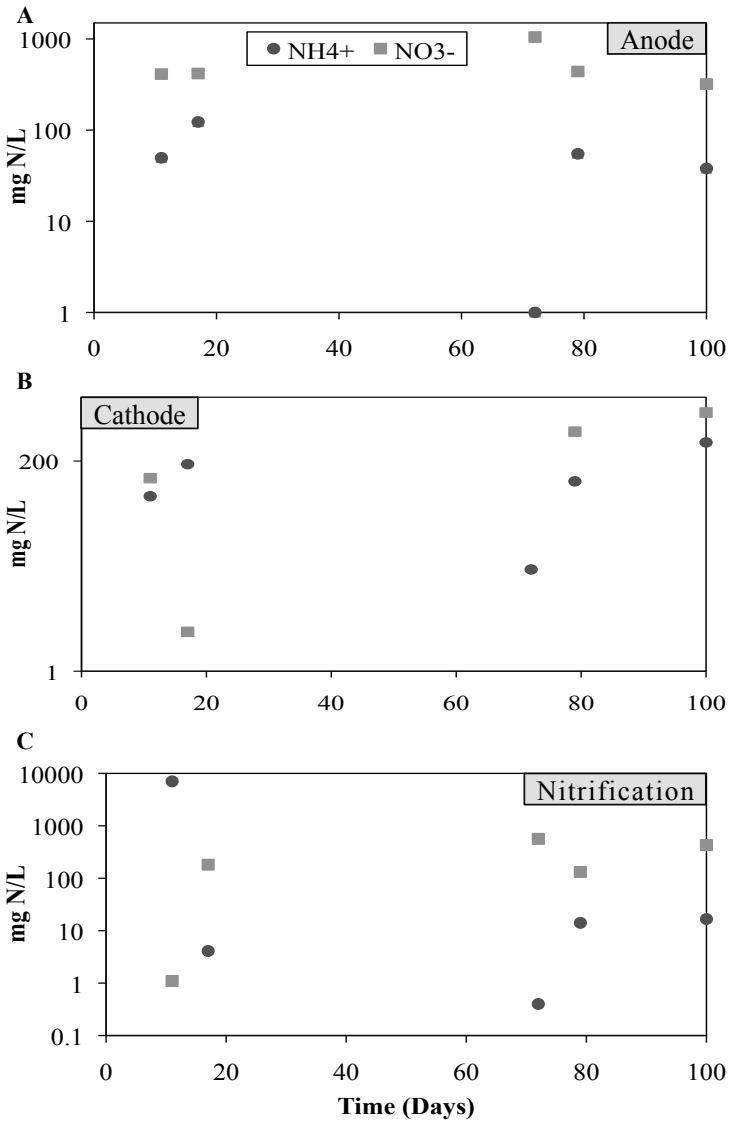

Figure 20. MFC Latrine performance. Ammonium and Nitrate concentrations in the anode (A), cathode (B), and nitrification chamber (C)

Due to the nature of the study, polarization curves to determine internal resistance could not be conducted on site. Power production was expected to be low due to high ohmic losses associated with large granular graphite electrodes and the complex nature of the waste compared to synthetic wastes often used in lab-based studies. On-going studies

in the laboratory are exploring MFC configurations that will yield improved power outputs.

There was evidence of nitrogen transformations in the MFC Latrine. Rates of nitrification and denitrification were not calculated due inconsistency of latrine use (i.e. not being able to measure a consistent fluid flow through the system) and infrequency of data collection. Nitrification in the nitrification chamber was indicated by low ammonium concentrations compared to the initial high reading (Figure 20C). Due to incomplete nitrification, ammonium accumulation was observed in the cathode (Figure 20B). Low ammonium concentrations and high nitrate concentration in the anode over time suggest that there is nitrification occurring in the anode, likely due to oxygen intrusion from the headspace in the incompletely filled chamber (Figure 20A).

There was also evidence of denitrification in the cathode indicated by low nitrate concentrations over time. However, during the school recess, nitrogen species accumulated in the nitrification and cathode chambers. Organics were indirectly monitored through turbidity but low fluid levels and small graphite particles in suspension likely impacted results. Additionally, after the first 6 months of operation, the charge unit on the data logger failed and our partners were unable to collect additional data while repairs to the instruments were made. Furthermore, user interface challenges began to interfere with the system performance.

4.3.3 Use and Maintenance

There were several user challenges that affected the performance of the MFC Latrine. One challenge was the improper disposal of waste paper. Our educational approaches were insufficient in communicating that it was desirable to put waste paper

into the toilet, which is contrary to the local convention of putting waste paper in a trash bin, usually located next to the toilet. We expected the absence of a trash receptacle would force users to put the waste paper into the toilet. Instead, waste paper was placed in the ash and woodchip bucket and in the urinal. Failure to add ash and woodchips prevented sludge stabilization in the composting chamber and thus decreased the carbon-to-nitrogen ratio of the sludge, reducing the overall quality of the compost. Further research is needed to understand these actions effect on MFC performance. Considerable woodchips and ash were added to the composting chamber in May of 2013 before the chamber was closed and the second chamber was opened.

The second consequence of improper waste paper disposal is preventing access to the urinal. Male urine is diverted to the nitrification chamber to convert ammonium to nitrate for the first step in nitrogen removal. When the urinal is not in use, nitrification does not occur and the nitrate reduction cannot occur in the cathode, preventing power production in the MFC. Likewise, replacement of toilet paper, ash and woodchips did not occur regularly over the year. Our partners were unable to locate the supply of toilet paper we left and scrap letter paper was often used. During the follow up trip, corrective actions were taken to improve the user interactions with the MFC latrine. A separate waste paper container was placed inside the latrine along side a container for woodchips. Basic instructions for urinal use and waste paper disposal were also written on the latrine wall in permanent marker.

General maintenance of the system was also an issue. Within the few months of operation, the MFC Latrine was found in an unacceptable state of cleanliness. Through email communication with faculty and follow-up with school administration, the interior

of the latrine was cleaned. Interactions with NYASTEC administration revealed that they were not invested into the long-term maintenance of the system because of a history of external groups establishing projects on school grounds with no subsequent communications or follow up trips. Our visits have reassured NYASTEC of our commitment to the school and its students.

4.3.4 Construction Costs for the Experimental Pilot MFC Latrine

By U.S. standards, the overall construction of the MFC Latrine was relatively low, costing less than $1000 for local materials and $1200 for labor. The graphite granules were an additional $1700, including fees for shipping and clearing customs. Since this is a pilot project, there was redundancy in labor and materials and we expect that if the system were to be reproduced costs could be significantly reduced. We are also exploring electrode alternatives that can be produced locally to reduce costs further. Biochar, a charcoal produced by the carbonization of biomass and typically used as a soil additive (Lehmann, 2007), is being investigated as a potential electrode alternative. Preliminary studies have shown that biochar can sustain an average maximum power of 338 mW/m^3 (unpublished data) in bench-scale MFCs.

The reported costs also included labor and supplies to make the structure complement the surrounding NYASTEC buildings. A solid wood door with a lock, an extended metal roof with rain gutters, and footings to prevent soil erosion were added to the latrine superstructure. Toilet seat covers and toilet paper holders were added in the interior in a fashion consistent with the new toilet facilitates constructed on campus. Pesticides were applied to the wooden components to prevent termite damage and the latrine superstructure was painted to match the rest of the campus buildings. The local

laborers were also paid top wages. Since the system was adapted to the local resources, costs to reproduce the system elsewhere will reflect costs for local labor and materials. Additionally, if an electrode material could be locally sourced, the remaining MFC components cost $100. Therefore, it would be economical to retrofit existing composting latrines.

4.4 Discussion

4.4.1 Power Production and Waste Treatment

The MFC Latrine produced a maximum of recorded value of 6.75 uW to power a LED light on the interior of the latrine. There is also evidence of nitrogen removal within the system. Previous studies have shown that carbon and nitrogen can be removed from the anode and cathode, respectively, in combination with a separate nitrification process, sustaining 34.6 W/m^3 (Virdis et al., 2008). A membrane-less MFC for total nitrogen removal produced 19 W/m^3 (Butler, 2009).

These bench-scale MFCs used acetate as their electron donor in the anode, which is easily oxidized by anode-respiring bacteria (ARB). In the MFC Latrine, complex substrates are the fuels that drive electricity production. Power production is directly related to the complexity of the substrate in the anode (Pant et al., 2010). With various organic substrates, microbial communities become diverse due to varying metabolic pathways. During anaerobic metabolism, fermentative bacteria that cannot use the anode electrodes as the terminal electron acceptor will instead use the substrates for fermentation and methanogenesis (Logan & Regan, 2006; Zhang et al., 2012). Proteins and carbohydrates that enter the anode of the MFC latrine are first hydrolyzed and utilized by fermenters to produce organics acids and hydrogen (Eastman & Ferguson,

1981; Parameswaran et al., 2010). They serve as electron donors for the anode-respiring bacteria that ultimately transfer electrons to the anode, creating electricity. In the presence of methanogens, acetate can also be used to produce methane gas, limiting its availability for anode-respiring bacteria. This reduces the amount of energy that can be recovered from the wastes as compared to MFCs were simple organics are often used as electron donors. On going studies are currently exploring this anode hierarchy.

Only a few large scale, field-tested MFCs have been demonstrated, with limited success. A pilot scale MFC consisting of 12 vertical tubular reactors, with a combined liquid volume of 1000L was constructed in Yatala, Queensland, Australia to treat a dilute brewery wastewater. It yielded low COD removal in the anode caused biofouling in the air-cathode due to oxidation of organics in the cathode influent. (Logan, 2010). Although air-cathodes have also been shown to produce higher power densities when coupled with membrane-less MFC reactors (Liu & Logan, 2004), they become impractical for use in reactors that treat complex material wastewaters because of oxygen diffusion to the anode and biofilm accumulation on the cathodes due to incomplete removal of organics in the anode. Cusick (2011) constructed a continuous flow pilot-scale microbial electrolysis cell (MEC) of 910 L (liquid volume) to produce hydrogen gas from treating winery wastewater in Oakville, CA. After an intensive start-up period that explored pH and temperature effects on power, it produced a maximum current density of 7.4 A/m^3 and evolved 0.19 ± 0.04 L/L/day of hydrogen. MECs require external power input, making these systems nearly impossible to implement in developing countries where power sources are already limited. The greatest obstacle for the MFC latrine is overcoming user

challenges. Even with improper waste disposal blocking the urinal, the MFC Latrine still produced power and with adequate use, it has the potential to for improved performance.

4.4.2 MFC Latrine as an Improved Sanitation Solution

The MFC Latrine is a viable solution for the dissemination of sanitation facilities in Ghana and other developing countries because it provides incentives that other improved sanitation systems do not. Pit latrines, ventilated improved pit latrines (VIPs) and composting latrines (i.e., EcoSan) are common improved sanitation facilities in the developing world (Mihelcic, 2009). While traditional pit and VIP latrines offer a sanitary method for disposal of excreta, their use does not produce other valuable by-products, such as compost. Composting latrines, by definition, produce compost, and this product has been leveraged as part of sanitation development programs. However, they do not produce electricity like the MFC Latrine. It is anticipated that the electricity and compost production from the MFC Latrine could be deployed in a similar sanitation development program but with the added benefit of the electricity providing additional income opportunities. Also, it is possible that simple monitoring equipment could be powered from the MFC and thereby provide valuable information to sanitation entrepreneurs.

The performance of the MFC Latrine is dependent on frequency of use and proper operation and maintenance. Users must have a social acceptance of ecological sanitation methods and their outputs (e.g., use of human compost as soil additives) for the MFC Latrine to be successful. Educational efforts are known to improve the understanding of sanitation, hygiene, and water at the local level (Ramani et al., 2012). This can promote awareness of sanitation problems and establish a demand for sanitation facilities like the

MFC Latrine. A market for the compost must also be indentified to successfully build a sanitation-as-a-business model for the MFC latrine.

4.5 Conclusions

A Microbial Fuel Cell Latrine was constructed in Nyakrom, Ghana. All materials were procured locally with the exception of granular graphite and the LED electrical circuit. All construction was executed with local labor, with construction taking approximately 2.5 weeks. The total cost of the MFC Latrine system was $3900; however, a large portion of this cost was related to the construction of a new latrine superstructure and the experimental nature of the system. It is estimated that the cost to add an MFC component to a previously constructed latrine system would be 95% less; assuming a suitable, local alternative for imported granular graphite was available.

Power production was directly correlated with latrine use. During the study period, power production increased from the point of start-up until NYASTEC began summer break. While school was in session, power production from the full-scale MFC was consistent with pilot study results. Power production was generally low due to high ohmic losses and the complex nature of the waste.

There were multiple user challenges that negatively affected the performance of the MFC. These included the improper disposal of waste paper, failure to stabilize waste solids in the composting chamber by adding ash and woodchips, and inconsistent use of the urine diversion system. Educational programming was somewhat successful at overcoming these challenges. Sustainable use of the latrine ultimately requires establishing good user habits and incorporation of the sanitation technology into the user community's typical social practices.

There was evidence of total nitrogen removal through the MFC Latrine. In general, the MFC Latrine was successful at decreasing the organic matter and nitrogen from the waste stream.

The MFC Latrine succeeded as a proof of concept demonstration that a continuous flow, two-chamber MFC with a separate nitrification stage can use human waste to produce electricity. As such, the MFC Latrine is a viable option as an improved sanitation solution. The MFC Latrine has advantages over other improved sanitation technologies because the MFC Latrine produces electricity in addition to compost, all without the need for additional electrical inputs or waste collection and transportation.

Conclusions and Future Work

Design and pilot a low-cost, large-scale MFC - A laboratory and in-situ MFC was successfully developed using minimal cost-prohibiting materials. A three chamber system approach was undertaken to accommodate for the high concentrations of ammonium present in human urine. Nitrogen and organic matter removal was observed during various operational conditions in Phase I before the MFC began treating synthetic feces and urine solutions during Phase II. During all of the operational conditions, COD removal was greater than 90%. Nitrate removal in Phase I reached up to 76.8 ± 7.1% while nitrogen removal during phase II was 68.4 ± 2.8 mg N/L. Power production reached an average 3.40 ± 0.01 nW/m^2 during the Phase I and decreased to 0.66 ± 0.02 nW/m^2 in Phase II. The design was validated in the laboratory and deployed in Ghana.

Investigate the breakdown of complex organic matter in the MFC anode – Volatile fatty acids were characterized in the anode. The presence of acetic acid, propionic acid and N-butyric would suggest that methanogens, acetogens, and fermentative bacterial communities developed within the anode. Coupled with the low power production and high organic matter removal, it is hypothesized that these bacteria are outcompeting the anode respiring bacteria. Further molecular analysis is required to determine what organisms thrive in the MFC.

Deploy an MFC coupled with a composting latrine for waste treatment in Ghana – The MFC latrine was successfully deployed in May 2012. The MFC design created and tested

in the lab was used to retrofit a newly built composting latrine in Ghana for the purpose of improving sanitation in a rural village and the performance was monitored for a year.

Assess the MFC Latrine use by local users in Ghana – Maintenance and general cleanliness were issues with the MFC Latrine. The major obstacle was communicating with the school where the MFC Latrine was constructed and to obtain data of its daily use. A follow up visit in May 2013 showed that the MFC latrine might not be as frequently used by male users due to various factors, such as the path to the latrine has been narrowed due to other construction projects nearby. Further assessment in Agona Nyakrom is required to determine the user demand for latrines and the acceptance of the MFC latrine as a viable improved sanitation system.

Future Work – Identification of the microbial community structure in the anode and cathode from the lab-based pilot system is needed to understand what particular organisms are degrading organics and transforming nitrogen. Further characterization of the nitrogen transformation in the nitrification stage and cathode is also required to account for the different nitrogen species. Further research will be conducted to assess whether pathogens can be removed in the MFC portion of the MFC Latrine rather than within the composting chamber. Lastly, focus will be placed on the user interface challenges in for the MFC Latrine in Ghana to address the impact on the MFC treatment viability.

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