ABSTRACT

Title of Thesis: EVALUATION OF TREATMENT AND RESOURCE RECOVERY POTENTIAL OF BIOELECTROCHEMICAL SYSTEMS TO DC WATER PROCESS STREAMS BY BENCH AND PILOT SYSTEM

Aaron Matthew Leininger, Master of Science 2019

Thesis directed by: Dr. Birthe V. Kjellerup, Dept. Civil and Environmental Engineering

Microbial fuel cell and microbial electrolysis cell systems were developed and tested with different wastewater process streams from DC Water Blue Plains Advanced Wastewater Treatment Plant. These biofilm-based systems provide an alternative to the conventional activated sludge system by oxidizing wastewater organics without the need for mechanical aeration. In bench-scale systems, the application of high-strength solids-dewatering wastewater as a feedstock was shown to increase both treatment energy savings and energy recovery. Current densities in meso-scale microbial electrolysis cells were 3.3 and 3.6 times higher when fed dewatering-filtrate or a blend of filtrate and primary effluent as compared to reactors operating with primary effluent. An integrated 800L pilot biocathode microbial fuel cell system was designed and constructed, and initial results are reported. Over the first 43 days of operation, the system averaged 15% removal of chemical oxygen demand and a load removal of $110\ \mathrm{g}/(\mathrm{m}^3 * \mathrm{day})$.

EVALUATION OF TREATMENT AND RESOURCE RECOVERY POTENTIAL OF BIOELECTROCHEMICAL SYSTEMS TO DC WATER PROCESS STREAMS BY BENCH AND PILOT SYSTEM

by

Aaron Matthew Leininger

Thesis submitted to the Faculty of the Graduate School of the
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Advisory Committee:

Dr. Birthe V. Kjellerup, Chair

Dr. Alba TorrentsDr.

Matthew D. Yates

Acknowledgements:

This work was supported in part by funding from DC Water and by Engie by award of the 2017 GDF-Suez Chuck Williams Memorial Fellowship

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Table 1. *Average influent WW characteristics of primary effluent feedstock in pilot-scale MFC studies discussed*

	Chemical Oxygen Demand (mg/L)	Total Nitrogen (mg N /L)	Conductivity	Dissolved oxygen (mg/L)	pH	Total Suspended Solids (mg/L)
Minimum Average	**118** (Hiegemann et al.)	**25.8** (Hiegemann et al.)	**770 µS/cm** (Feng et al.)	**3.3** (Zhang et al.)	**7** (Zhang et al.)	**56** (Zhang et al.)
Maximum Average	**300** (Feng et al.)	**48** (Feng et al.)	**4.2 mS/cm** (Hiegemann et al.)	N/A	**7.3** (Hiegemann et al.)	N/A

Table 2. *Energy benefit summary of continuous flow single-chamber MFCs operating under realistic conditions with real WW. Energy benefits based on a theoretical large WWTP () and normalized to energetic activated sludge requirements for COD removal described in Wan et al. 2016.*

Reference	Size(L)	Design	Media	HRT (hours)	Average COD (mg/L)	Average COD Removal	Energy Savings (kWh/day)	Maximum NER (kWh/m^3)	Energy Recovery (kWh/day)	Net Energy Benefit (kWh/day)	Energy Recovery / NEB	CE (%)
Zhang 2013	4	Tube	Primary Effluent	11	279.7	68%	170416	0.024	23900	194316	0.123	13.2
Feng 2014	250	Stackable Panel	Raw WW	144	300	79%	212352	0.054	54000	266352	0.203	5
Hiegemann 2016	45	Cassette	Primary Effluent	22	118	24%	25375	0.012	12000	37375	0.321	24.8
Dong2015	90	Cassette	Brewery WW	72	815	86%	628006	0.056	56000	684006	0.082	19.1
Dong 2015	90	Cassette	Brewery WW	144	3321	88%	2618542	0.097	97000	2715542	0.036	8
Park 2017	0.15	Flat-panel	Primary Effluent	0.5	144	34%	43868	0.008	8400	52268	0.161	12

Chapter 2

*Table 1. Cathode open-circuit potentials. Values are means and standard deviations (n=3) in millivolts (mV) vs. reference (Ag/AgCl). * Day 18 values are of the initial OCP; all other values are after at least 24 h at the OCP.*

MFC	Day 0	Day 6	Day 14	Day 18*	Day 27	Day 46
Mn coating	237.3 ± 8.0	159.3 ± 30.1	159.0 ± 38.8	48.3 ± 29.1	198.3 ± 23.1	129.0 ± 68.4
Pt coating	293.3 ± 81.5	144.7 ± 12.6	241.7 ± 29.1	233.0 ± 47.6	277.7 ± 17.0	211.3 ± 9.1

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Table 1. Summary of treatment and energy recovery from second fill cycle of operational phase (□□□□ = □□□Ω) closed circuit duplicate averages and open circuit (OC)

MFC Feedstock	COD Removal (mg/L)	COD Removal (%)	Total Charge Passed (Coulombs)	Coulombic Efficiency (%)	Volumetric Energy Recovery (kWh / m^3)
Filtrate	602 ± 492	16 ± 13	35	2	0.0017
Filtrate OC	120 ± 229	3 ± 6.2	N.A	N.A	N.A
Primary	138 ± 10	62 ± 4	33	7	0.0016
Primary OC	143 ± 8	64 ± 3	N.A	N.A	N.A
60/40	400 ± 72	25 ± 5	39	3	0.0023
60/40 OC	-100 ± 248	-6 ± 15	N.A	N.A	N.A
80/20	295 ± 24	32 ± 3	22	2	0.0007
80/20 OC	292 ± 16	32 ± 2	N.A	N.A	N.A

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Table 1. Overview of reported pilot system performances and the current study

Reference	Size(L)	Design	Media	HRT (hours)	Average COD (mg/L)	Average COD Removal	Energy Savings (kWh/day)	Maximum NER (kWh/m^3)	Energy Recovery (kWh/day)	Net Energy Benefit (kWh/day)	Energy Recovery / NEB	CE (%)
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Park 2017	0.15	Flat-panel	Primary Effluent	0.5	144	34%	43868	0.008	8400	52268	0.161	12
This Study	800	Brush Module Cassette	Primary Effluent/ Filtrate Blend	24	882	15%	1180000	.00000621	6.21	**1180000**	**~0**	N.D.

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Table 1: Summary of MEC performance during second displayed fill cycle (day 6-13) with influent filtrate (1567±11 mg/L) and primary effluent (122±9 mg/L) soluble COD

MEC Feedstock	sCOD Removal (mg/L)	sCOD Removal (%)	Total Charge Passed (Coulombs)	Coulombic Efficiency (%)	Hydrogen in headspace?
Acetate	447 ± 7	55 ± 1	11104	59	Y
Filtrate	283 ± 117	18 ± 7	5371	45	N
Filtrate OC	13 ± 86	1 ± 6	N.A	N.A	N
Primary	68 ± 6	56 ± 5	1643	57	N
Primary OC	79 ± 6	65 ± 5	N.A	N.A	N
60/40	150 ± 66	21 ± 9	5883	93	N
60/40 OC	-23 ± 75	-3 ± 11	N.A	N.A	N

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Chapter 1

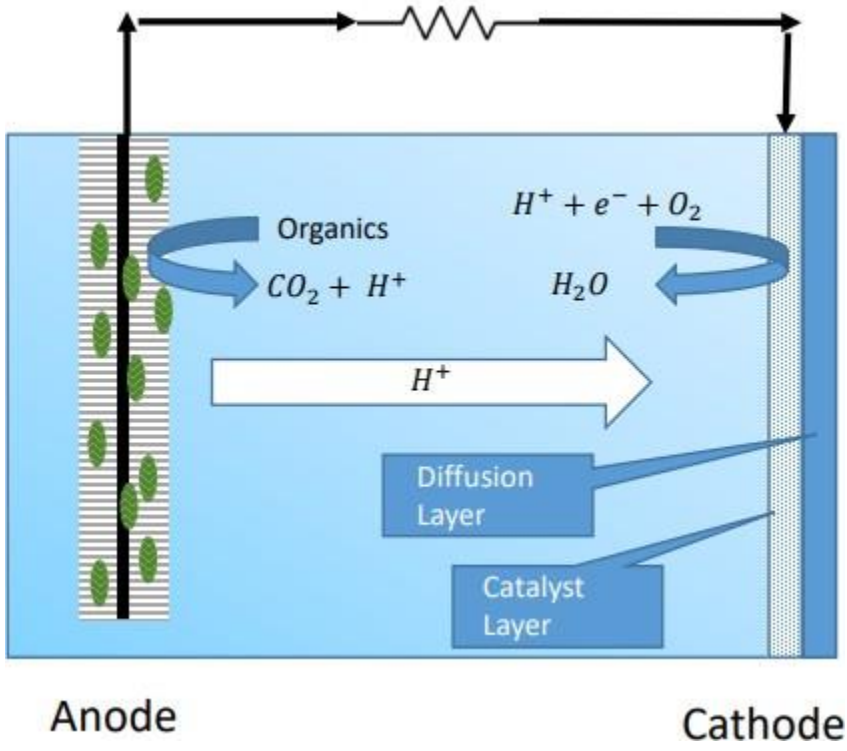


Figure 1. *Simplified schematic of a single-chamber, membrane-less air cathode MFC.*

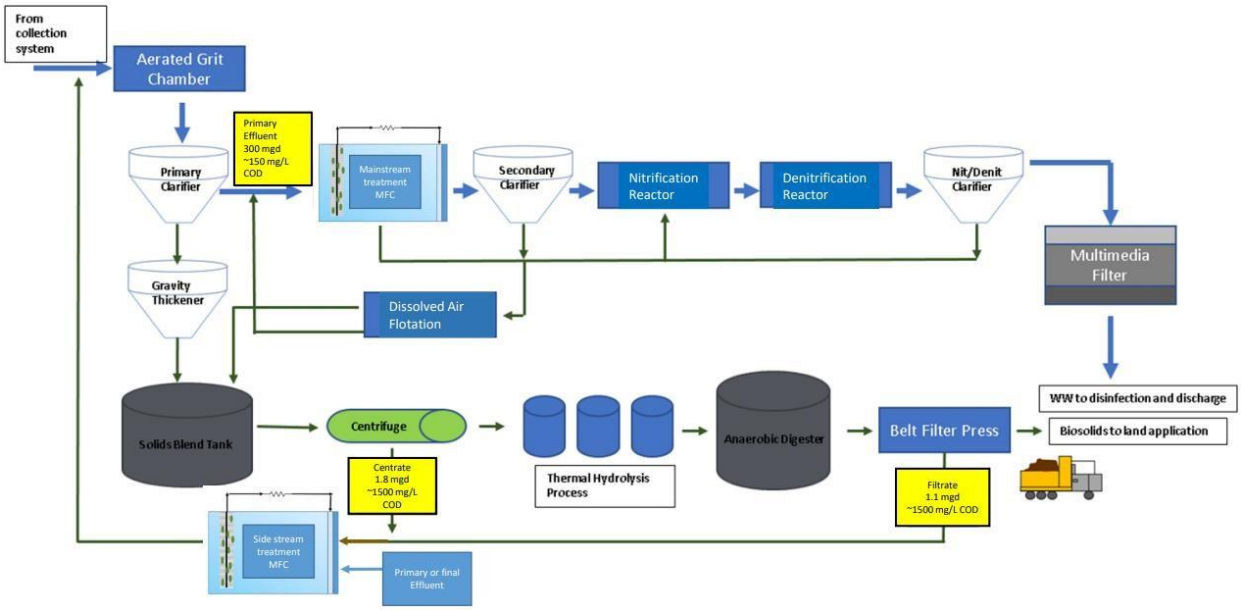


Figure 2. *Simplified process diagram of an advanced wastewater treatment plant with MFC relevant process streams highlighted*

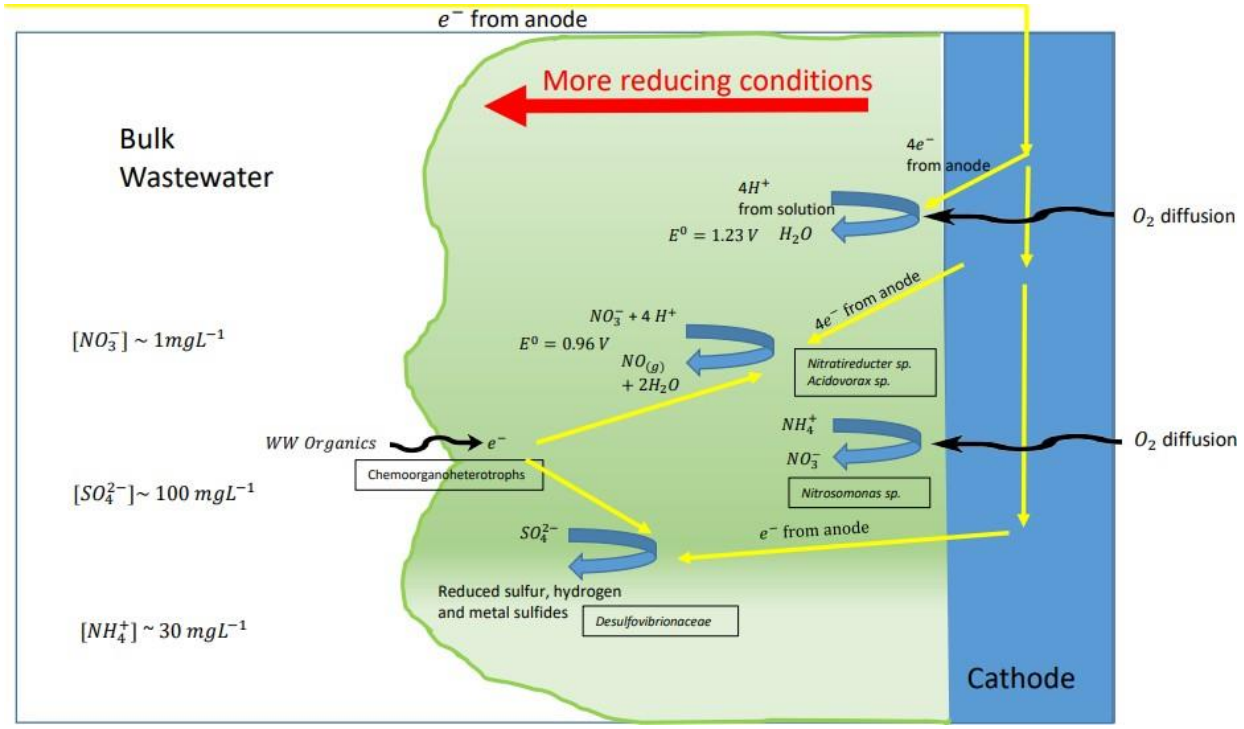


Figure 3. *Competing and complementing reduction/oxidation reactions observed on cathodes. Thickness, conductivity, and community structure of cathodic biofilm may affect oxygen concentration gradients and redox potentials, leading to localization of processes.*

Chapter 2

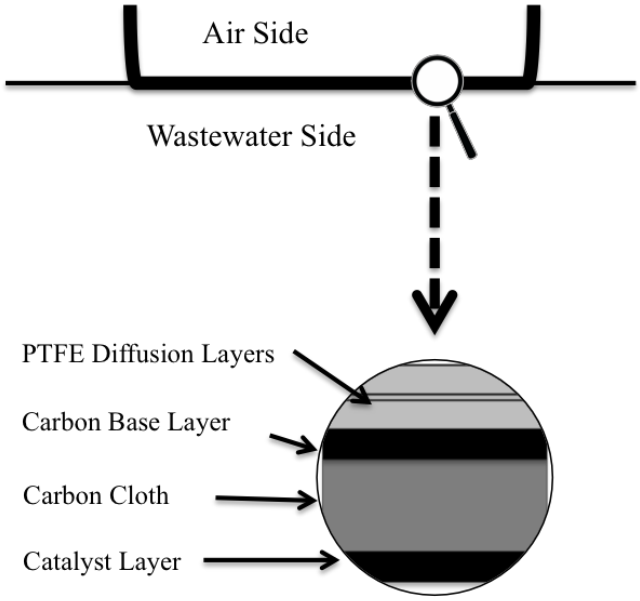


Figure 1. Cross-section of a floating air cathode and close-up of the cathode layers.

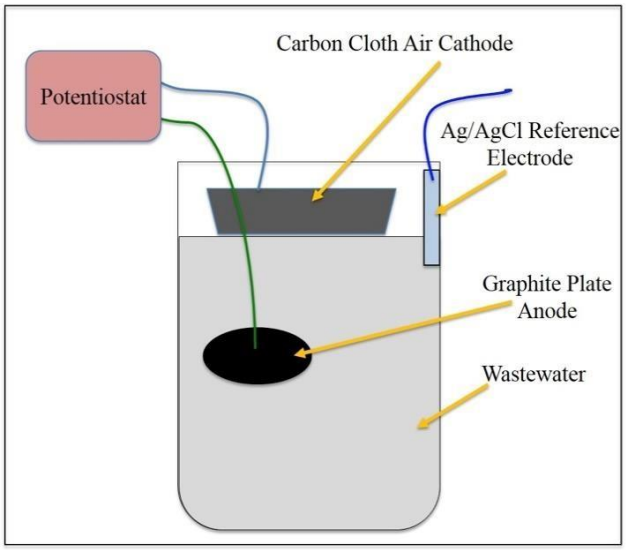


Figure 2. Laboratory-scale wastewater-sediment microbial fuel cell.

Figure 3. Four of six MFCs in the three-electrode configuration.

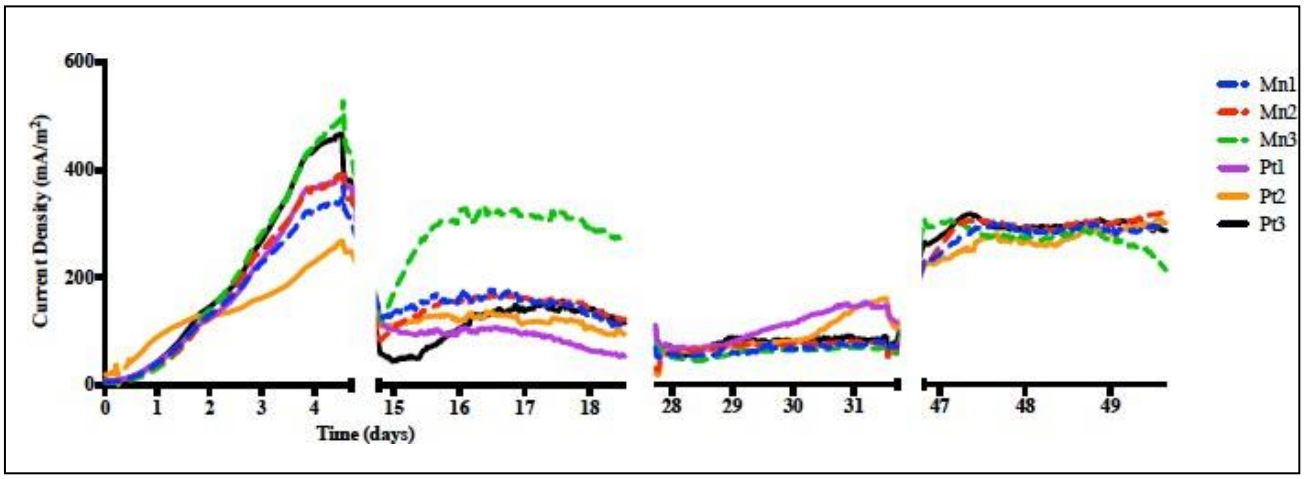
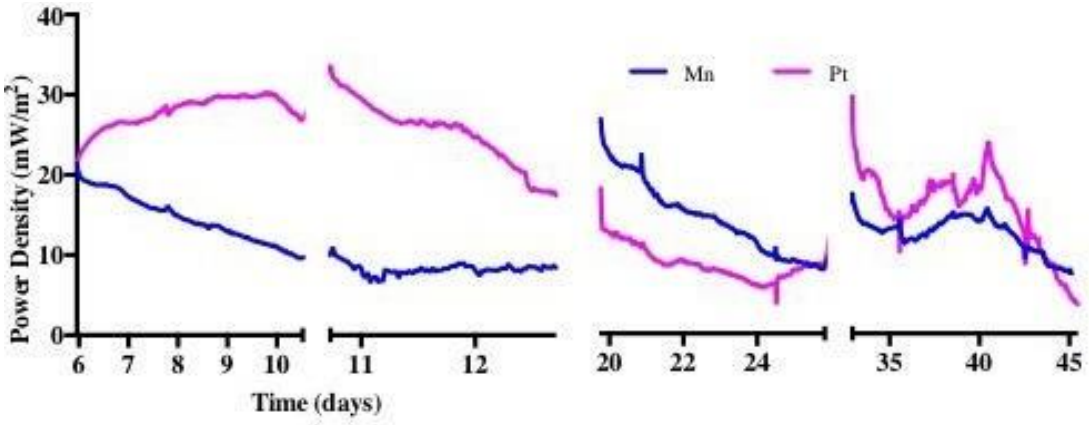


Figure 4. Current density in MFCs operating in the three-electrode configuration with the anode as the working electrode, the cathode as the counter electrode, and an Ag/AgCl reference electrode. Anodes were poised at −0.2 V *vs*. the reference electrode. The results were normalized to the anode surface area.



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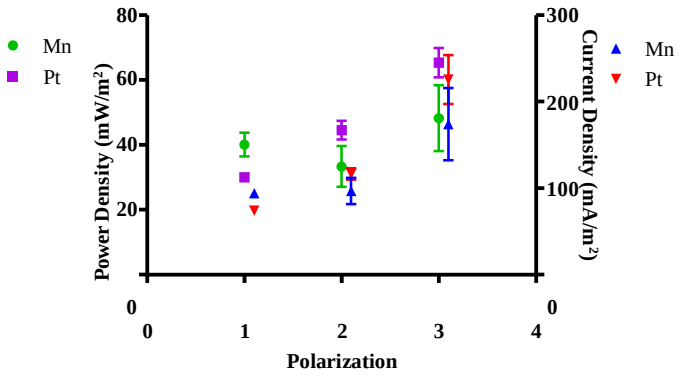
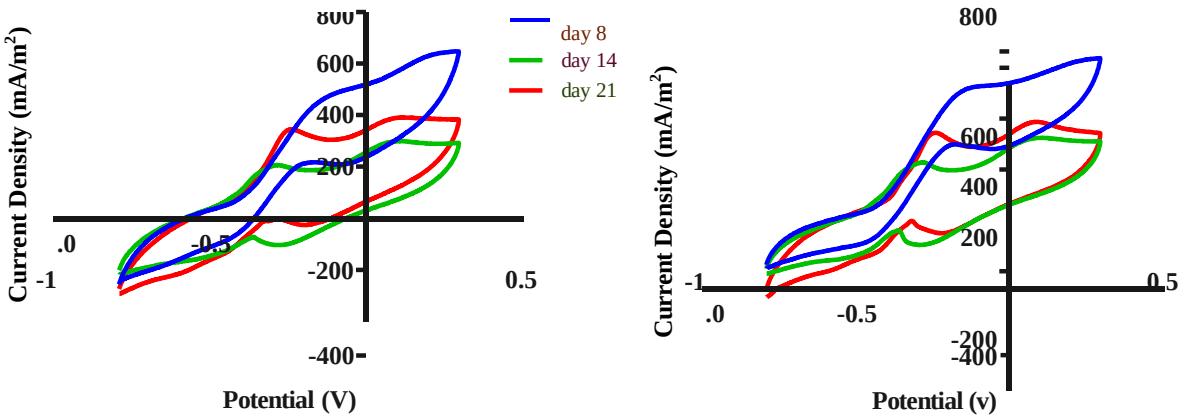


Figure 6. Average and maximum power values and the current densities at which they were reached for both types of cathode (error bars show one standard deviation).



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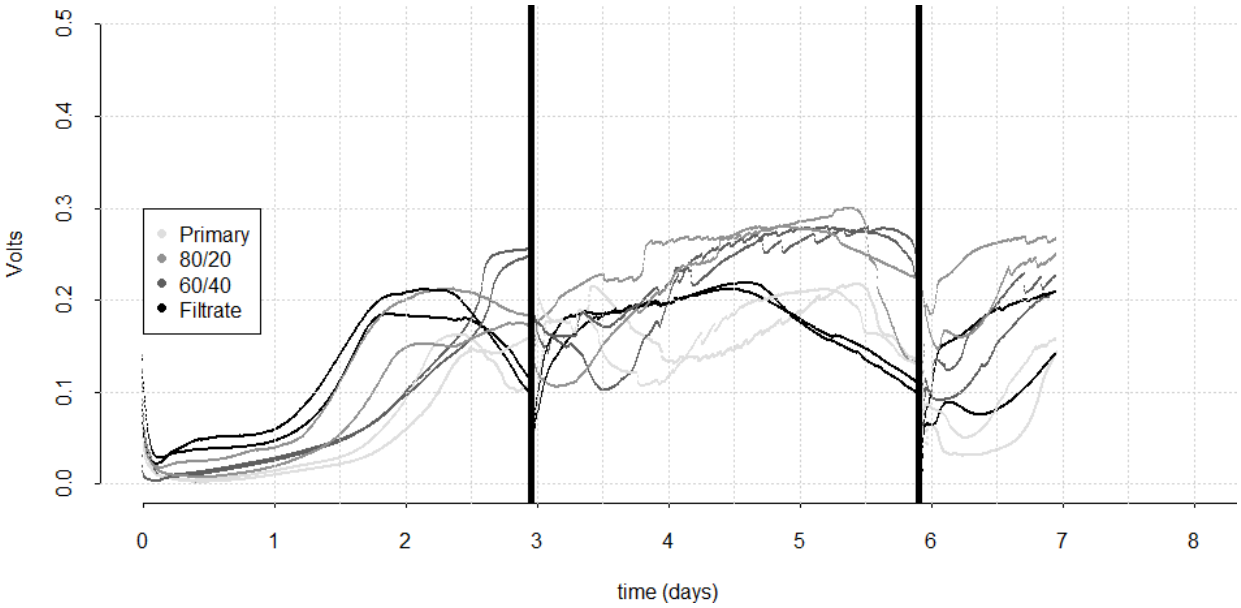

Fig 1. MFC startup performance and polarization of different blends during first seven days of operation and first three fill cycles , □□□□ = □□□□□Ω

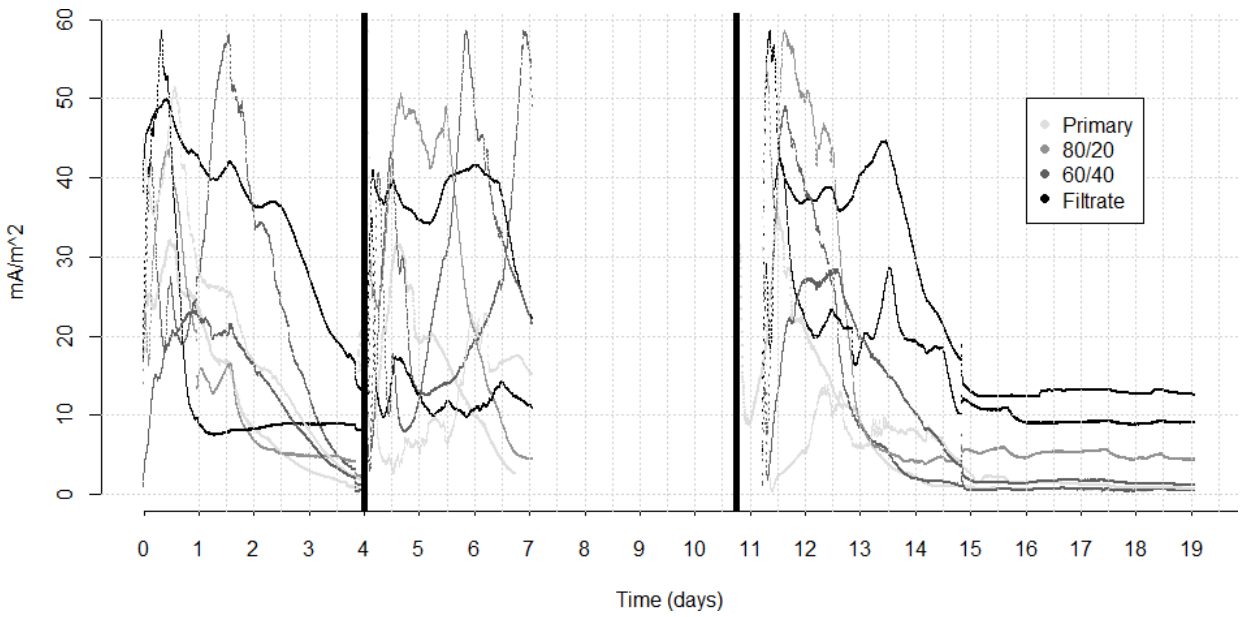

Fig 2. Current density of different wastewater blends in first three fill cycles after R_{ext} = 1000Ω

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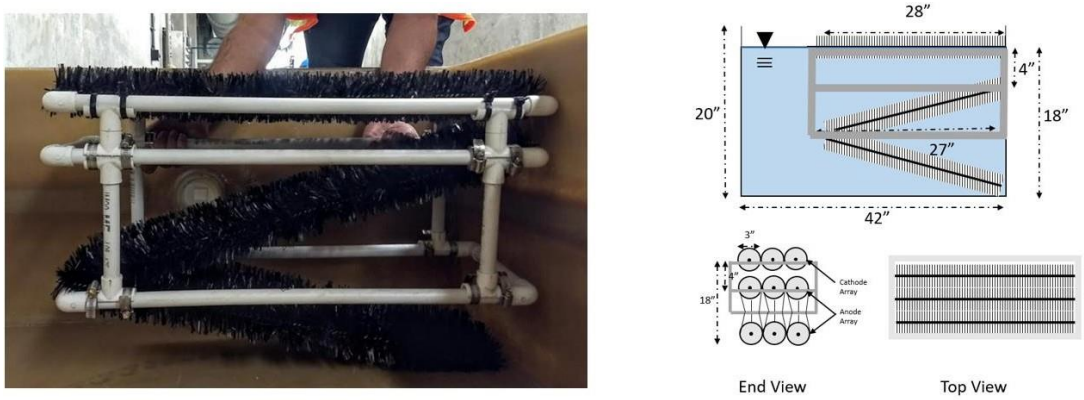
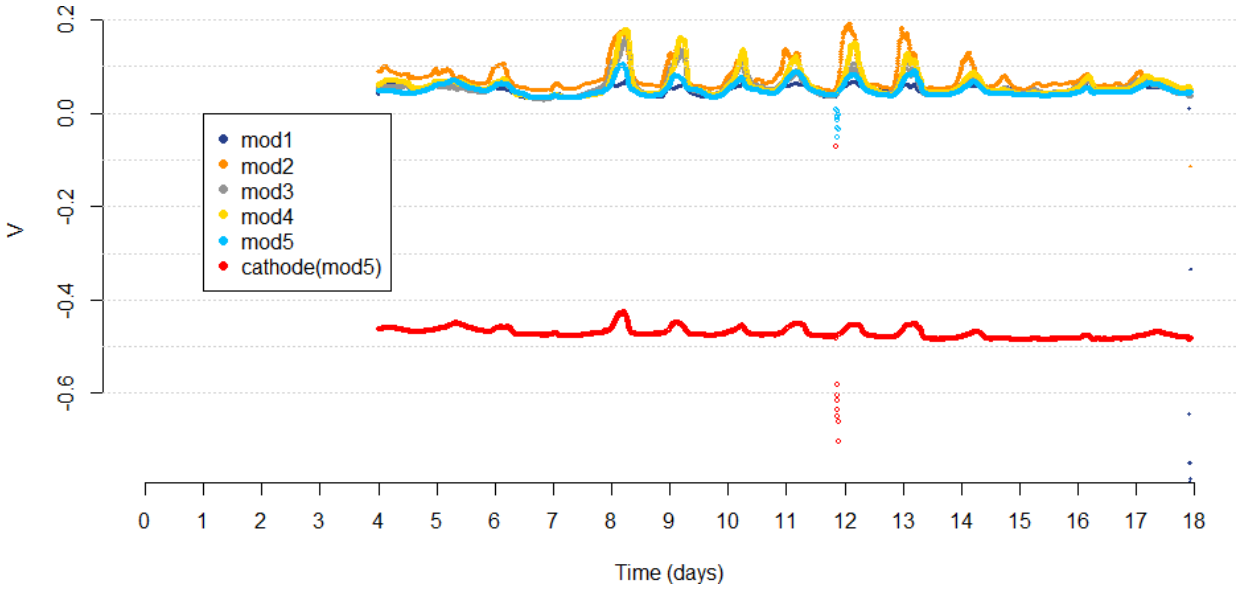


Figure 1: Design and layout of a single MFC module



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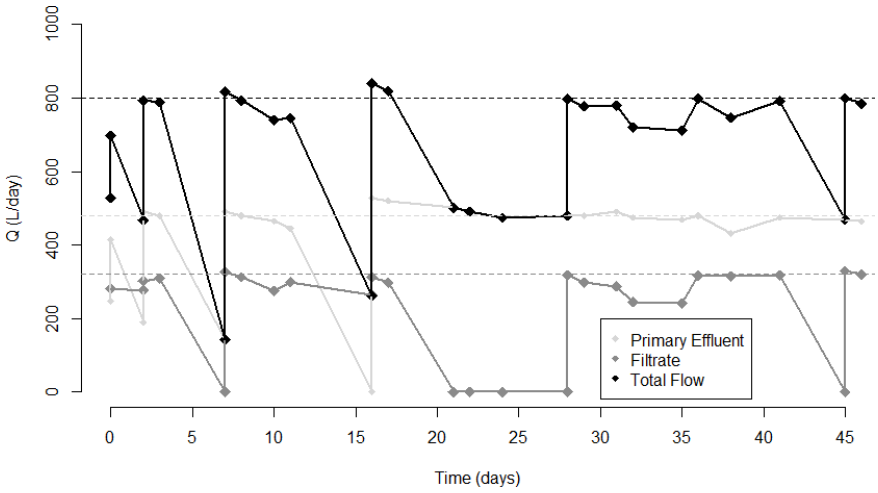

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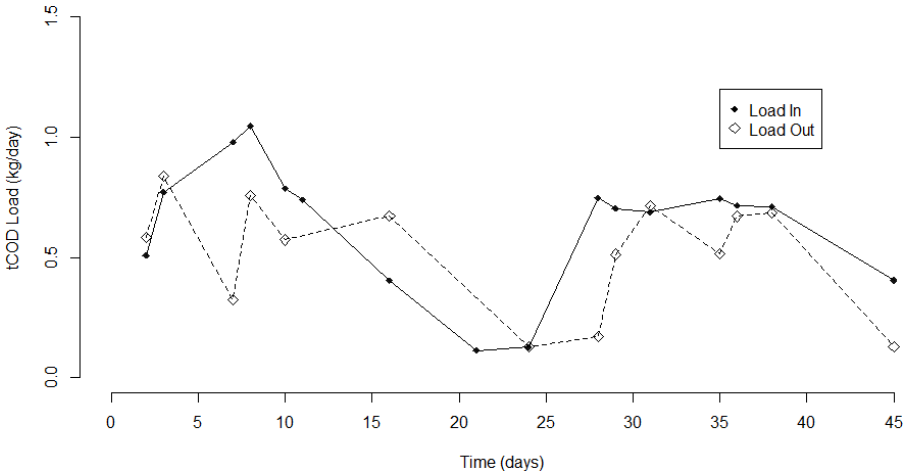


Fig4. tCOD loading and removal during first 45 days of closed-circuit operation (Oct 8 - Nov20). Days 0-41 across Rext = 1000Ω, Days 42-45 across Rext=15Ω. Will add second axis with Total Kjeldahl Nitrogen Loading once data is analyzed

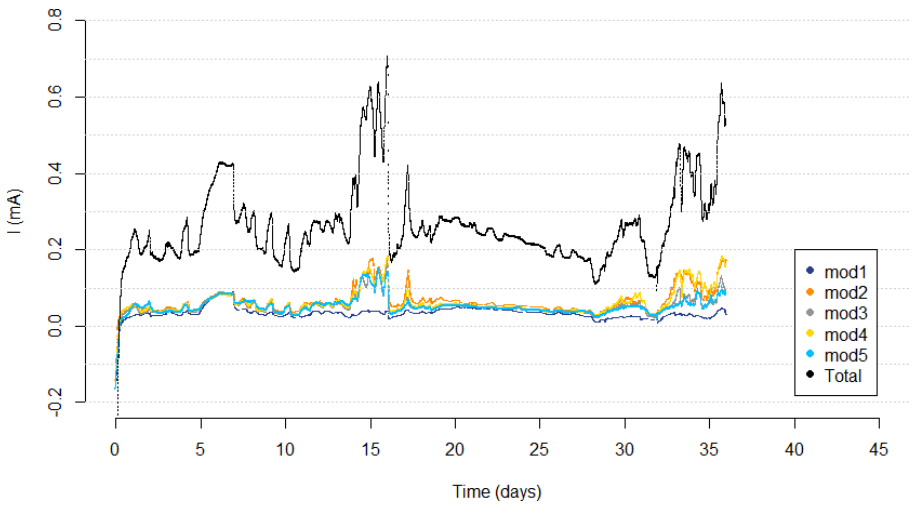
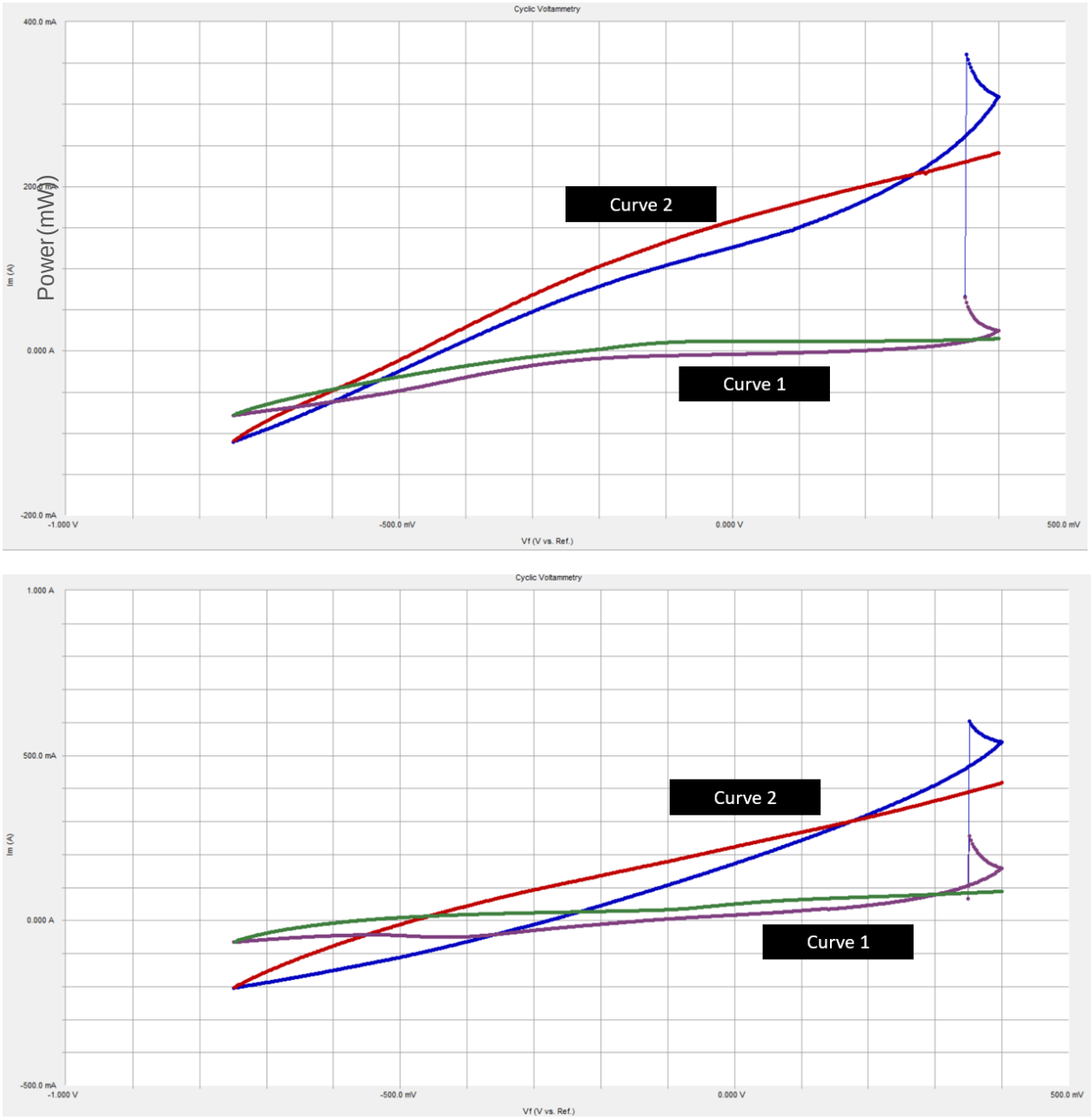


Fig5. Current output of MFC modules during first 36 days of closed-circuit operation (Rext = 1000Ω)



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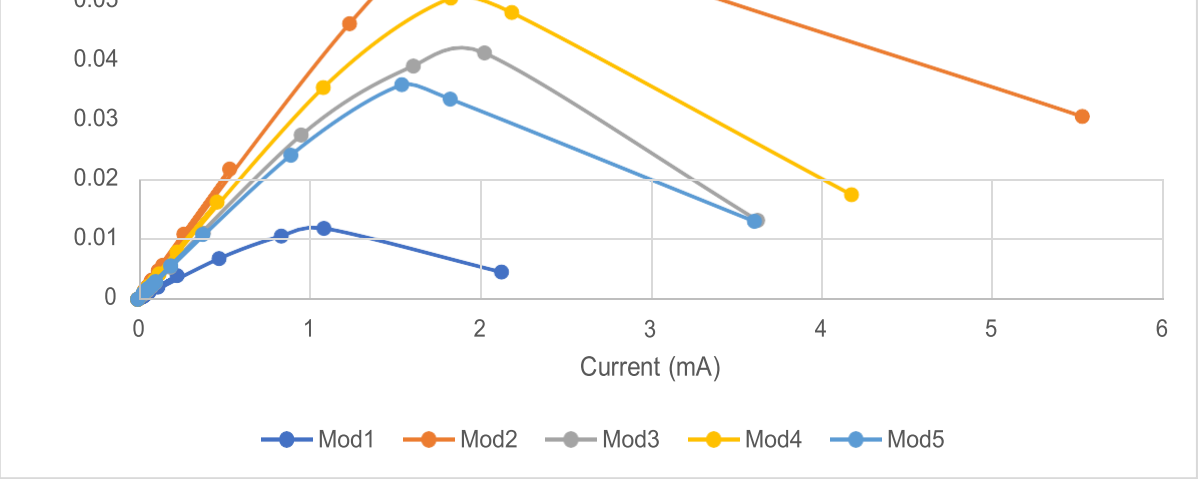

Fig7. Overlay of power curves from all modules, created after 29 days of operation across 1000Ω external resistor.

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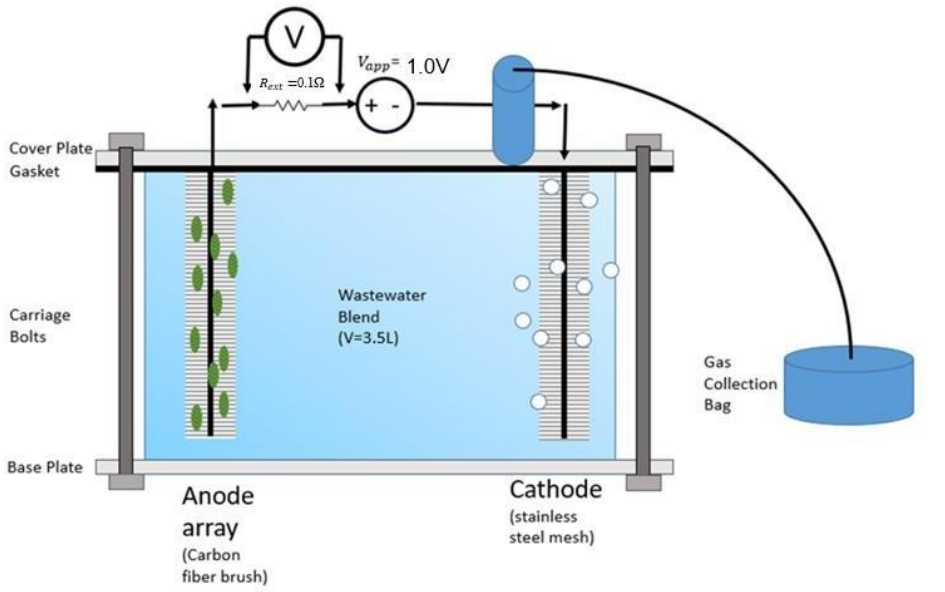

Fig1. Single-chamber MEC used in study

Chapter 1

Implementation of upscaled microbial fuel cells for optimized net energy benefit in wastewater treatment systems

Aaron Leininger[1], Wing-Mei Ko[1], Mark Ramirez[2], Birthe Kjellerup[1]

[1] University of Maryland, College Park Department of Civil and Environmental Engineering

[2] DC Water Blue Plains, Resource Recovery, Washington DC

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Abstract

Microbial fuel cells are bio-electrochemical devices that use exoelectrogenic biofilms to convert organic matter into electric energy. Recent research of microbial fuel cells has occurred because of their potential to perform energy net-positive degradation and transformation of wastewater constituents. However, most studies have focused on small scale (<1 L) batch systems with simple and homogenous feedstocks such as acetate. These studies are useful for identification of isolated variables in a controlled environment, but the system architectures are not scaleable to wastewater treatment applications and the feedstocks do not reflect the heterogeneity of real domestic wastewater. The emerging research evaluating scaled-up microbial fuel cells operating with domestic wastewater substrate has described new and different biofilm characteristics and associated transformations other than those seen in bench studies. Scale-up of wastewater microbial fuel cells presents challenges including feedstock variability, changing environmental conditions, and biofouling. To advise the design of pilot-scale systems, this review discusses process stream selection and system architecture with the aim to maximize net energy benefit, defined as the sum of treatment energy savings and direct energy recovery.

Introduction

Wastewater treatment plants (WWTPs) rank among the largest point users of energy in many municipalities. Estimates show that energy consumption at WWTPs amounts to 1.5% of all electricity produced in the United States (Logan 2008). According to U.S. EPA, the expected demand for electricity at the facilities will grow at 0.8% annually in the next 25 years (Energy Information Administration (U.S.) 2015). This highlights the need for innovation in energy-effective and environmentally sustainable wastewater treatment. Despite the large amount of energy required to treat wastewater for organic matter and nitrogen, wastewater can also become a source of energy, since it contains as much as nine times the amount of energy that is required to treat it (Shizas and Bagley 2004). Wastewater is mainly composed of water and a variety of dissolved and suspended organic and inorganic materials (McCarty et al. 2011). The main organic constituents of domestic wastewater include carbohydrates, lipids, and proteins (Raunkjær et al. 1994). The energy content of wastewater is measured using chemical oxygen demand (COD), which describes the amount of oxygen needed to oxidize the organic matter into carbon dioxide and water.

Large amounts of chemical energy are contained in these molecules as chemical bonds and estimates of the caloric energy content range from 15-29 $\square\square * \square^{-1}_{\square\square}$ (Heidrich et al. 2011). In the US alone, each person produces on average 378 l of wastewater per day (USEPA 2015) resulting in approximately 44 trillion liters annually. The energy content in this volume of wastewater corresponds to 1-2% of the annual electricity consumption in the US (Shizas and Bagley 2004; U.S. EPA 2015). While wastewater contains large amounts of energy as measured by COD,

conventional treatment processes expend large amounts of energy to remove the organic content. Aeration processes designed to oxidize and thereby remove biodegradable organic matter (measured as Biological Oxygen Demand (BOD) or COD) during secondary treatment can account for up to 60% of the energy utilized in the WWTP (Menendez 2010). In the US, 21 billion kWh was used in 2001 for aeration during secondary treatment (Goldstein and Smith 2002).

Microbial fuel cells (MFC) are based on a technology that creates useable energy through the oxidation of energy-rich organic materials present in wastewater through microbial processes occurring in biofilms (Logan 2008; Zhang et al. 2013; Paitier et al. 2017). Biofilm-based, or attached growth systems have advantages over conventional activated sludge treatment because of their higher treatment intensity and more stable and robust microbial communities (Ødegaard et al. 1994, Andreottola et al. 2000, Ahmed et al. 2017). Unlike other biofilm-based systems such as moving bed bioreactors or rotating biological contactors, MFCs have the potential to achieve wastewater treatment goals of BOD/COD and solids removal while simultaneously recovering energy in the form of electricity. This is in contrast with conventional wastewater treatment processes, which use energy to treat the wastewater without recovering the energy already contained herein. Water treatment efficiencies of pilot-scale MFCs have varied between 24-79% for COD, 40-50% for suspended solids, and 28-76% for total nitrogen (Zhang et al. 2013; Feng et al. 2014; Hiegemann et al. 2016) with hydraulic retention times (HRT) less than 24 hours. Improvements in system architecture have also led to the demonstration in nitrification and denitrification within MFCs, providing discharge satisfactory nitrogen removal from wastewater in HRTs of several hours (Park et al. 2017b). MFCs offer the possibility to supplement or

supplant traditional treatment processes such as aerated activated sludge systems used for removal of organic material and nitrification. This could make wastewater treatment an energy-neutral or even net positive process by eliminating the need for energy intensive aeration. More broadly, MFCs play a role in the ideological shift from "Wastewater Treatment" towards "Resource Recovery".

Research in wastewater treatment applications of MFCs is a relatively new topic in environmental engineering that have taken off in the past 10-15 years (Kim et al. 2003; Rago et al. 2019). Currently there are no major WWTPs incorporating large-scale MFC units into their treatment processes. MFC studies have mainly used small, bench-scale reactor systems (Figure 1), where "artificial wastewater" or simple homogenous feedstocks were applied (Cheng et al. 2006; Dong et al. 2015; Al-Mamun et al. 2017). This review will discuss characteristics of organic matter and wastewater process streams, microbial community composition and function, and net energy benefit with the goal of informing the design of scaled-up.

Wastewater organic material

Anodic biofilms are dynamic communities that facilitate the oxidation of organic matter and production of mobile electrons. Biofilm composition and/or system performance are responsive to the influx of anolyte (Ghadge et al. 2016), changes in feedstock (Jadhav and Ghangrekar 2009), temperature (Ahn and Logan 2010) and environmental conditions (Mohan et al. 2008; Jadhav and Ghangrekar 2009). Enhancement of the microbial processes is critical for successful application of MFCs for wastewater treatment (Karra et al. 2013; Park et al. 2017a). An ideal

MFC system for wastewater treatment should have a resilient microbial community that can withstand varying environmental conditions and still be actively producing electricity (Jadhav and Ghangrekar 2009).

Inoculation of MFC's with wastewater or digester solids is a common practice used to introduce desired microbial communities into an MFC during start-up (Ishii et al. 2012; Karra et al. 2013; Ghadge et al. 2016). Anode respiring bacteria (ARB) such as *G. sulfurreducens* can readily metabolize acetate and yield mobile electrons (Lovley 2008; Ishii et al. 2012). However, domestic wastewater contains large and complex organic molecules that require hydrolysis prior to oxidation by many ARBs and exoelectrogenic bacteria (Lovley 2008). An effective anodic biofilm in a wastewater MFC should contain a diverse group of microorganisms that are capable of anodic respiration, hydrolysis and fermentation. To facilitate this, a multi-step feedstock acclimation process has been proposed. MFCs that were pre-acclimated by successive acetate and glucose feedstocks showed higher current density and coulombic efficiency than identical non-acclimated wastewater MFCs (Park et al. 2017a). Pyrosequencing results showed that biofilm diversity was higher in non-acclimated systems than acclimated systems as measured by Chao and Shannon indices (Park et al. 2017a). This low diversity on the acclimated anodes indicated that selected anode respiring and glucose fermenting bacteria formed stable and competitive communities. However, there were not significant differences in the COD removal rates between the systems, suggesting that non-ARBs were also active in COD removal and that the wastewater contained consortia of microorganisms capable of both hydrolysis and exoelectrogenesis (Park et al. 2017a). This suggests that pre-acclimation of a system may be important for improving energy recovery, but not significant to the treatment potential of an

MFC system. Several studies have shown that the soluble COD (SCOD) was degraded more readily than total COD (TCOD) in MFCs (Huang and Logan 2008; Zhang et al. 2013). This was consistent with the expectation that dissolved forms of carbon would be more readily available to microbial communities than particulate forms in situations where the electron acceptor would not be limiting. When selecting a process stream for MFC treatment, it is therefore advantageous to select a stream where most of the COD is dissolved.

Process stream evaluation

MFC application for wastewater treatment focuses on removal of organic matter and nitrogen from a process stream to reduce operating costs associated with aeration (Feng et al. 2014; Park et al. 2017b) and direct energy recovery. Currently, most pilot-scale wastewater MFC research has focused on raw wastewater influent or primary effluent (Zhang et al. 2013; Feng et al. 2014; Hiegemann et al. 2016) because of high levels of readily available organics and the ability to provide treatment early in the process train (Figure 2). However, further research should investigate other locations along the treatment pathway to maximize the net energy benefit. The positive relationship between the COD concentration in MFC feedstock and energy recovery (Wang et al. 2014; Santos et al. 2017) suggests that other process streams from solids dewatering providing more concentrated flows with higher organic matter content could greatly improve the energy recovery. These high strength wastewaters could be utilized by themselves or as an optimized blend with primary effluent or treated plant effluent. Additionally, because these process streams are typically returned to the head of the plant, removal of COD or nutrients from these highly concentrated process streams could provide large energy savings and improve concentration-dependent reaction kinetics. At WWTPs using thermophilic hydrolysis to

circumvent biological hydrolysis prior to anaerobic digestion, the feedstock potential of supernatants or filtrates after this process may be even greater because of the presence of highly bioavailable carbon as cell lysis material.

Temperature and feedstock conductivity have been shown to have a positive relationship with energy recovery potential in continuous flow systems in the temperatures ranging from 13-29 °C and solution conductivities from 1.5-6 mS/cm (Hiegemann et al. 2016). These relationships can be justified by improved microbial kinetics under higher temperatures and lower ohmic losses from proton transport in more conductive electrolytes. In WWTPs using inorganic coagulants such as ferric chloride, high strength wastewaters from biosolids processing have concentrated levels of dissolved solids, making them more conductive, and often with an elevated temperature because of thermophilic treatment or digestion. While raising the temperature or ionic strength of the main wastewater stream may be impractical and uneconomical by direct means (i.e. adding heat and salts), blending high strength wastewaters with the main process stream may provide the same benefits of improving reaction kinetics and reducing ohmic losses from proton transport.

One of the problems with utilizing biosolids dewatering process streams is the high (>800 mg/L ($NH_3 + NH_4^+$)) concentration of ammonia, which can inhibit MFC performance (Nam et al. 2010; Kim et al. 2011; Tice et al. 2014; Hiegemann et al. 2016; Hiegemann et al. 2018). In a pilot-scale wastewater MFC study, the feedstock changed from primary clarifier effluent to the supernatant from a sludge pre-thickener (Hiegemann et al. 2016). The biofilms experienced a shock load of free ammonia (50 ± 10 mg/L NH_3) and the power density of the MFC dropped to zero. When the previous feedstock was reestablished, the MFC restored the power density

within two days. Studies have reported varying thresholds for free ammonia concentrations above which performance inhibition is seen, ranging from 11 mg/L (Nam et al. 2010) to 64 mg/L (Hiegemann et al. 2018), suggesting that environmental conditions or biofilm characteristics may play a role in the ability of electroactive biofilms to tolerate ammonia. However, results have shown that the same concentration (50 ± 10 mg/L) would not inhibit activity if the anode biofilms were given time to acclimate (Kim et al. 2011). Since free ammonia-nitrogen is hypothesized to be more important for performance than ammonium (NH_4^+) (Hiegemann et al. 2018), the pH may be an important and easily adjustable parameter to control ammonia speciation. Controlling system pH to be significantly below the acid dissociation constant of ammonium (9.24) would minimize free ammonia and therefore associated inhibition of anodic respiration. Despite claims that biosolids-dewatering process streams are unsuitable as MFC feedstocks (Hiegemann et al. 2018), ammonia levels of high strength wastewaters could be mitigated by dilution or amendment with primary effluent or other process streams and may provide a significantly greater net energy benefit than primary effluent alone.

Oxygen reduction reaction catalysts and the cathodic biofilm

One of the greatest barriers to development of large-scale MFC systems is the cost and performance of the cathode. The microbial communities that form on cathode surfaces are less understood than anodic biofilms but may be as important as anodic biofilms because the oxygen reduction reaction (ORR) occurring on the cathode is often the limiting process in MFC function (Clauwaert et al. 2007b; Rismani-Yazdi et al. 2008). Chemical cathode catalysts such as platinum and metal oxides are effective at increasing the rate of ORR and improving energy recovery but are expensive and are subject to fouling or degradation over time (Logan 2010).

Since the purpose of the cathode is to provide a site for and facilitate the reduction of oxygen, a biofilm that encourages or catalyzes ORR is desirable (Xia et al. 2013). While formation of cathodic "fouling" is unwanted (Logan 2010; He et al. 2016) and cleaning with commercial bleach and hydrochloric acid solutions has led to increased performance (He et al. 2016), in several cases the formation of biofilms on cathode surfaces was associated with performance improvements (Cheng et al. 2006; Xia et al. 2013.) Some biocathodes can utilize terminal electron acceptors other than oxygen such as nitrate or sulfate thus reducing the need of the rate-limiting ORR and in some cases promoting nitrogen removal through nitrate reduction (He and Angenent 2006; Logan 2010; Park et al. 2017b). Improved performance of biocathode functions and management of detrimental biocathode activity is one of the most important challenges to scale-up of MFCs.

The biofilm in the "near cathode region" is complex and may host many processes because of the diversity of electron donors such as wastewater organics, metabolites, and anodic electrons and electron acceptors such as oxygen, nitrate and sulfate (Figure 3). Electrode separators, commonly used to prevent direct contact of electrodes, can also impact cathodic biofilm communities (Rago et al. 2018). A greater understanding of the functions and activities of cathodic biofilms is needed to develop a design and management strategy.

The conventional nitrification/denitrification pathway of nitrogen removal that oxidizes ammonia nitrogen to nitrate and then reduces nitrate to nitrogen gas is among the most inefficient and redundant processes at WWTPs, involving cost-intensive aeration, introduction of

a carbon source such as methanol for denitrification and the production of biosolids that subsequently must be managed (Du et al. 2007; Virdis et al. 2008). While MFC applications to wastewater treatment most commonly target treatment of organic compounds and energy production, they can also simultaneously transform and/or remove nitrogen without the need for aeration (Clauwert et al. 2007a; Feng et al. 2014; Park et al. 2017b). For an acetate-fed MFC that utilized a biocathode, denitrification proportional to the current production occurred thus suggesting a molar ratio between the oxidation of organics in the anode biofilm and the use of nitrate as a terminal electron acceptor at the cathode biofilm (Clauwaert et al. 2007a). Nitrogen removal has also been observed in wastewater studies. Here, a bench-scale hybrid MFC-membrane bioreactor system achieved over 97% TN removal from primary clarifier effluent (30 $□□ \cdot □ \cdot □^{-1}$) at hydraulic retention time (HRT) ranging from 48-72 hours (Malaeb et al. 2013). In another study, a flat-panel MFC system achieved over 90% TN removal of primary clarifier effluent and a TN removal rate of 0.62 kg-N/m^3/d with an HRT of only 2.5 hours (Park et al. 2017b). In this study, satisfactory effluent nitrogen levels (<2 mg*L^{-1}) at a low HRT were obtained by using very large air cathodes ($400 □^2 / □□□h□□□ □^3$) to promote oxygen transfer and nitrification. Denaturing gradient gel electrophoresis showed the presence of both aerobic nitrifying and anaerobic denitrifying bacteria near the MFC cathode. Since the near-cathode region is a zone of transition between aerobic and anoxic conditions, it is a key area for nitrogen transformation, and the observation of co-existing nitrifying and denitrifying bacteria in this region was important. While nitrogen removal most often has not been considered as a primary goal of MFC implementation, it is an important process occurring within MFCs that could prove to be an effective application of the technology. Further work on the pilot scale is needed to understand how system design and biofilm management influence the electrochemical

performance and denitrification capacity of biocathodes. It may be most efficacious to have different designs to fit different niches within the plant's framework thus some MFCs could be optimized for removal of organic material and others optimized for removal of nitrogen.

The ability of a biocathode to effectively catalyze ORR can be influenced by the feedstock composition and system configuration. In one study using acetate, the performance of an MFC with a biocathode in single and double-chamber configuration was compared to that of MFCs using traditional platinum catalysts (Xia et al. 2013). In the double-chamber configuration, where the cathode was not exposed directly to the feedstock because of an anion exchange membrane, the biocathode performed as well as or better than the platinum catalyzed cathode (554 ± 0 $□□□^{-2}$ vs 576 ± 16 $□□□^{-2}$). However, when the biocathode was used in a single chamber MFC, where the biocathode was directly exposed to the feedstock, the performance declined and the platinum catalyzed cathode outperformed the biocathode (1253 ± 45 $□□□^{-2}$ vs 199 ± 0 $□□□^{-2}$) (Xia et al. 2013). The investigators attributed this result to either heterotrophic microorganisms outcompeting the oxygen reducing biocathode microorganisms for oxygen or the cathodic microorganisms shifting their electron source from cathode electrons to acetate (Xia et al. 2013). However, analysis of the microbial communities present at the biocathode was not performed thus it is not known how the populations differed between the environmental conditions. This study was conducted using an acetate feedstock, which would have been readily available to heterotrophic microorganisms that colonized the cathode surface as a carbon and electron source. One of the conclusions of this study is that effective biocathodes should use a separator between the biocathode and high concentrations of organic materials (Xia et al. 2013). Separators (Rago et al. 2018) and membranes (Xu et al. 2012) are known to foul and diminish

MFC performance as well. In a wastewater system containing electron donors more recalcitrant than acetate, it is possible that heterotrophic bacteria would not dominate the desirable, electron accepting autotrophic microorganisms to the extent purported in this study.

Net energy benefit

MFCs provide direct energy recovery as electricity and treatment energy savings. Estimating the net energy benefit of an MFC system is system-dependent and requires consideration of the effects on downstream processes. For example, replacing aerobic secondary treatment with MFCs could reduce the volume of biosolids and associated dewatering, stabilization, and transportation costs, but could also decrease the yield of biogas from an anaerobic digester (Hiegemann et al. 2016) if solids digestion is used at the WWTP. Some MFCs could also provide substantial treatment energy savings from nitrogen removal (Malaeb et al. 2013; Park et al. 2017b) which may be dependent on process stream selection and system design and operation. A simplified approach to estimating the net energy benefit (NEB) of a system intended to function in place of secondary treatment incorporates only the secondary treatment energy savings and direct energy recovery (EQ-1). The treatment energy savings (EQ-2) is based on the COD removal of the MFC system applied to the main process stream of a plant and an average of 3.2 $□□ * □_{□□}^{-1}$ expended by aeration to remove COD in the conventional activated sludge process (Wan et al. 2016). The energy recovery (EQ-3) is estimated based on the volumetric normalized energy recovery ($□□□_{□}$) of the MFC system applied to the flow rate of the main process stream.

EQ-1: □□□ = □□□□□□□□□□ □□□□□□□ □□□□□□□□ + □□□□□□□ □□□□□□□□□

EQ-2: □□□□□□□□□□ □□□□□□□□□□□□□□ = □□ □ −) ∗ □ ( ) ∗ 3.2 □□ □□□ ∗ 0.002778 □□ ℎ □ ∗ % □□□□ − □□□

EQ-3: □□□□□□□□□□□□□□□□ = □□□□ □ (□□ □3) ∗ □ (□3 □□)

In this approach, where only secondary treatment savings and direct energy recovery are considered, the value of treatment energy savings has far outweighed the value of direct energy recovery in all reported pilot-scale studies (Table 1). Direct energy recovery has only been 1.4-6.5% of the net energy benefit of implementation in pilot-scale domestic wastewater MFCs. This suggests that with the current technology, MFC implementation should be viewed primarily as a low-energy treatment process.

However, scaled-up wastewater MFCs have great potential for improvement of the energy recovery based on the low ratio of exoelectrogenic COD degradation to total COD degradation, defined as coulombic efficiency (CE), observed in the literature. CE of bench-scale MFCs over 50% are not uncommon (Fan et al. 2007; Zhang et al. 2015), but pilot-scale MFCs fed with domestic wastewater have shown lower CE ranging from 5 to 25% (Zhang et al. 2013; Feng et al. 2014; Hiegemann et al. 2016). This may in part be attributed to the presence of microbial activity on suspended wastewater flocs. While it has not been systematically examined, the ratio of suspended to attached electrode growth in an MFC system may be related to the system CE. A study by Zhang (2015) showed that feedstock COD and CE were inversely proportional during a lab scale experiment (Zhang et al. 2015). Since higher COD wastewater streams may

allow suspended growth to proliferate, lower CE may be expected but the treatment energy savings may be greater than with an MFC from the main process stream. While CE is valuable to a point in understanding a system, the net energy benefit of a system should be the primary metric used to evaluate and compare systems (Table 1).

Recommendations for MFCs in wastewater treatment

Design of MFC systems aimed at long-term operation should focus on membrane-less or biocathode systems that are cost effective and reduce problems associated with biofouling (Hiegemann et al. 2016). Since the ORR often is the limiting reaction in MFCs, the MFC design should enhance the cathodic surface area, incorporate low-cost chemical or biological catalysts, and/or foster a catalytic cathodic biofilm (Cheng et al. 2006; Xia et al. 2013; Logan et al. 2015). Maintaining a sufficient cathode specific surface area will be a challenge as reactors increase in size, and the electrode packing density should be balanced with the potential for clogging (Logan et al. 2015). U-shaped anode-cathode module systems (Dong et al. 2015; He et al. 2016) that have an air cathode offer the benefit of high electrode packing density but may be limited by water pressure on the cathode diffusion layer (Ahn and Logan 2014). The modules in these studies have shown to be effective in tanks that were 12.5 (He et al. 2016) and 20 (Dong et al. 2015) cm deep, but reactor tanks at WWTPs are often at least 5 m deep. Therefore, it will be a challenge to allow passive oxygen diffusion to a cathode through an air-water interface at high water depths and in larger tanks. A scaled-up version of a rotating, drum-shaped cathode, similar in design to a rotating biological contactor (Pynaert et al. 2003), may be a solution for allowing passive oxygen diffusion at tank depths and hydrostatic pressures associated with real WWTP reactor systems, but has not been tested at a pilot-scale.

Another major consideration in the design of a pilot-scale system is biosolids management. wastewater streams contain large amounts of suspended biosolids, which MFCs remove through interception and settling (Dong et al. 2015). Biosolids accumulation in the anodic regions can promote methanogenesis and reduce power production due to transfer of electrons in the biosolids instead of the MFC (Ghadge et al. 2016). This suggests that a large scale MFC application may require periodic emptying of the settled material and subsequent cleaning or an integrated biosolids collection system. Module or stackable systems allow for easy servicing, removal of biosolids to control sludge blanket depth, or management of electrode biofilms (Feng et al. 2014; Dong et al. 2015).

Conclusion

The potential of MFCs to provide energy-effective and sustainable treatment of wastewater is large and the exploration is just beginning through pilot-scale studies (Feng et al. 2014; Dong et al. 2015; Hiegemann et al. 2016). These studies have shown that continuous flow MFCs are capable of effective treatment of wastewater containing organic matter, nitrogen, and solids through the actions of diverse and resilient microbial biofilm communities. However, detailed microbial analyses of biofilms have not previously been included in pilot-scale MFC studies, so it is unknown how environmental conditions, variations, and feedstock selection impact biofilm composition and transformations and in turn system performance.

While not discussed in this paper, other bioelectrochemical systems such as microbial electrolysis cells (MECs) may be an effective alternative or supplement to MFCs in wastewater treatment and energy recovery. In MECs, where an additional voltage is applied in order to create conditions for cathodic reactions other than oxygen reduction such as the reduction of protons to hydrogen, the system architecture is not limited by the need for an air-water interface at the cathode. This may allow for more intensive electrode packing than is possible with air-cathode MFCs and application to different process streams, such as MEC enhanced anaerobic digestion (Asztalos and Kim, 2017).

Further work should focus on understanding the behavior of electroactive biofilms under operational conditions in WWTPs to inform design and to improve the economic feasibility of scaled-up MFC systems. This work should also include considerations on making MFCs scaleable and viable systems that fit into specific roles within the greater WWTP framework such as replacement of secondary treatment, concentrate treatment, nitrogen removal, or other applications which serve to improve the energy balance of water infrastructure.

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Chapter 2

Electricity Generation from Wastewater Using a Floating Air Cathode Microbial Fuel Cell

Maia Tatinclaux[1], Kyla Gregoire[1], Aaron Leininger[1], Justin C. Biffinger[2], Leonard Tender[3], Mark Ramirez[4], Alba Torrents[1], and Birthe V. Kjellerup[1]*

[1] Department of Civil and Environmental Engineering, University of Maryland, College Park, MD

[2] Chemistry Department, University of Dayton, Dayton, OH. 45469

[3] Center for Bio/Molecular Science and Engineering, Naval Research Laboratory, Washington, DC

[4] Blue Plains Advanced Wastewater Treatment Plant, DC Water, Washington, DC

Published research work, Water-Energy Nexus

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Abstract

Recovering energy from wastewater is an important frontier of environmental engineering and science. Of the many proposed strategies, microbial fuel cells (MFCs) provide a direct path to electricity generation. Here, we report MFCs equipped with floating carbon-cloth air cathodes modified with manganese oxide (MnOx) or Platinum nanoparticle oxygen reduction catalysts. The performances of these MFCs were compared using domestic wastewater in a configuration suitable for electricity generation from primary settling tanks. The open-circuit voltages of the Mn-MFCs decreased gradually over time while those of the Pt-MFCs remained stable indicating that Mn leaching from the electrodes was occurring. Over 90% of the MnOx catalyst was solubilized from the cathode surface within the first two weeks of operation. Initially, the Pt-MFCs did not generate as high of a current density as MnOx but after 55 days, Pt-MFCs had a higher average maximum power density during polarization than Mn-MFCs: 65.4 ± 4.6 and 48.4 ± 10.16 mW/m^2 (based on anode geometric surface area), respectively. These results show the importance of evaluating promising alternative MFC cathode catalyst like MnOx in actual wastewater since it is difficult to predict how new catalysts designed to decrease cost yet increase the efficiency of the reduction of oxygen will respond in real-world wastewater applications.

Introduction

Wastewater treatment plants (WWTPs) are often the largest consumers of energy in their areas, accounting for approximately 4% of the electricity used in the US and other developed countries (E.P.A. 2013, Graham-Rowe 2012, Network 2002). The most widely used wastewater treatment method in the US is the activated sludge process, which utilizes mechanical aeration to facilitate oxidation of organic matter (the primary function of treatment) by aerobic bacteria (Oh et al. 2010). This process forgoes capturing the energy liberated by the reaction, which is dissipated as heat. As much as 60% of the energy consumed by a WWTP is due to aeration, accounting for an estimated 21 billion kWh/y in the US alone (Goldstein and Smith 2002). In contrast, wastewater contains significant amounts of organic matter, which, if the primary treatment were eliminated, would provide 14.7 kJ/g-chemical oxygen demand (COD) of energy (Shizas and Bagley 2004). A large advanced WWTP on the east coast of the US with a flow of 300 million gallons per day (MGD) would generate 38.7 MW per day (assuming an organic content of 200 mg/L wastewater) compared to the 20 MW required for the mechanical aeration of this volume. The large amount of energy required for wastewater treatment by standard methods represents a major obstacle to its realization in the developing world; thus, the ability to harvest energy directly from wastewater treatment would be transformative.

Microbial fuel cells (MFCs) produce electricity from microbe-catalyzed oxidation of organic matter at the anode coupled to reduction of oxygen at the cathode and are proposed as a potential waste-to-energy technology (Logan et al. 2006). There are two primary MFC configurations. In dual-chamber MFCs, an ion-exchange membrane is used to separate the anode in the anodic half-cell from oxygen in the cathodic half-cell while maintaining ionic conductivity between the electrodes; both of which are necessary for electricity generation. In single-chamber MFCs, an air-permeable cathode (air cathode) is used to seal an opening in the MFC, which contains the anode. In this configuration, the cathode has access to oxygen on one side and ions in the anolyte on the other, with oxygen (ideally) completely consumed as it permeates

the cathode. Unfortunately, wastewater readily fouls ion-exchange membranes and air cathodes, greatly diminishing the performance of both (Baturina et al. 2012, Dong et al. 2013, Martin et al. 2011, Nagahara et al. 2008, Sleutels et al. 2009). As a result, recent studies on the scale-up of wastewater systems have focused almost entirely on single-chamber MFCs (Dong et al. 2015, Hiegemann et al. 2016, Park et al. 2017).

In this study, we evaluated whether the primary settling tanks of WWTPs could be configured as large single-chamber MFCs. Due to intrusion of oxygen from the overlying air and settlement of particles, the top 1–3 mm are partially oxygenated and have a low solids content. Therefore, it may be possible to float a cathode on the wastewater surface, which would minimize exposure of the cathode to wastewater (Baturina et al. 2012, Dong et al. 2013, Nagahara et al. 2008) while providing access to oxygen from air without requiring the cathode to act as a physical seal to retain the anolyte (*i.e.*, wastewater).

In this study, we evaluated laboratory-scale MFCs consisting of 2 L open-top beakers filled with wastewater collected from a WWTP primary settling tank. Each MFC was equipped with a carbon cloth-based air cathode that floated on the wastewater surface, and a graphite anode positioned deeper in the wastewater. In addition, performance of β-MnO_2 (MnOx) as an oxygen-reduction reaction (ORR) catalyst was evaluated in comparison with conventional platinum nanoparticle ORR catalysts. While MnO_x catalysts have shown promise in both double and single chambered MFCs (Dai et al. 2015, Huggins et al. 2015, Li et al. 2010, Roche et al. 2010, Zhang et al. 2009), these catalysts have only recently been tested in domestic wastewater by Kharkwal and co-workers (Kharkwal et al. 2017), where manganese dioxide catalyzed cathodes were used e in biological oxygen demand (BOD) biosensors that functioned over a period of 1.5 years. In another application, MnOx cathodes resulted in a 13.5% increase in power density compared to bare carbon cathodes recently as part of a constructed wetland MFC reactor designed for the treatment of oily wastewater (Yang et al. 2016). Here, the membrane-less MFCs equipped with floating air cathodes using primary wastewater as the source of carbon achieved relatively high current and power densities.

Materials and Methods

2.1 Manganese Oxide Catalyst Preparation

β-MnO_2 (MnOx) particulate catalyst were immobilized generated on Vulcan Carbon using a published protocol (Huggins et al. 2015) which was modified from a method reported by Roche and Scott (Roche and Scott 2009). In brief, 4 g of Vulcan Carbon (VC) XC-72 [Cabot, Boston, MA] were suspended in 100 mL of a 10 mM $MnSO_4$ [Sigma-Aldrich, St Louis, MO] aqueous solution in a 500 mL one-neck flask. The VC was sonicated for 15 min at room temperature to assure that it was evenly dispersed. A magnetic stir bar and reflux condenser were added prior to heating the slurry at 80°C for 30 min. An aqueous 114 mM solution of $KMnO_4$ [Sigma-Aldrich] was added through the reflux condenser over 5 min with heating to maintain the temperature. The reaction was refluxed for 30 min and filtered hot through a GV 0.2 µm filter [Pyrex, Tewksbury, MA] and washed with warm deionized (DI) water. The resulting solid was dried in vacuum at 100°C for 12 h. The final yield was 3.9 g (99%) and the loading of MnO_2 to carbon was approximately 20% (wt. percent).

2.2 Electrode Construction

The anode was comprised of a flat, circular graphite plate [Mersen® G10], geometric surface area of 15.4 cm^2, that was sonicated in DI water three times for 15 min each. A press-fit electrical connection was made using a nylon screw, nut, washer, and titanium wire [McMaster-Carr®, Princeton, NJ]. The cathode (**Figure 1**) was constructed by applying oxygen diffusion layers to Panex 30 High Purity Carbon Cloth [Zoltek, St. Louis, MO] based on an published protocol (Middaugh et al. 2008). The carbon cloth used was not preliminarily wet-proofed and two, rather than the recommended four, layers of 60% polytetrafluoroethylene (PTFE) [Sigma-Aldrich] were applied. This was to reduce the internal resistance created by the PTFE layers, as two wet-proof layers were adequate to allow the carbon cloth to float on the wastewater surface. The edges of the PTFE-coated carbon cloth were then folded up and affixed using

superglue, resulting in a boat-like shape with the diffusion layer facing the air and the carbon cloth serving as the base. These cathodes floated on the wastewater surface with a wetted surface area of 21 cm^2. Next, the carbon-cloth side was coated with a layer of catalyst. The catalytic layer of 0.5 mg MnO_2/cm^2 or 0.5 mg Pt/cm^2 was prepared using 3.33 mg of 20% MnO_2/VC powder or 15% Pt/VC [FuelCell Earth©, Stoneham, MA] per cm^2 cathode surface area. The MnO_2/VC powder was mixed with 0.83 µL DI water, 10 µL Nafion™ solution [Ion Power, New Castle, DE], and 3.33 µL isopropanol per mg MnO_2/VC powder used. The mixture of these ingredients was sonicated with glass beads for 1 h and the resulting catalyst ink was magnetically stirred for 4–6 h to ensure a homogenous composition. Finally, the catalyst ink was evenly applied to the wetted cathode surface using a paint brush (procedure adapted from (Middaugh et al. 2008)). A press-fit electrical connection at the edge of the cathode was made using a nylon screw, nut, washer and a plastic-coated titanium wire.

2.3 Sampling and Experimental Setup

Six MFCs, referred to as Mn1, Mn2, Mn3, Pt1, Pt2, and Pt3, where three MFCs had MnO_2/VC cathode coatings and three had Pt/VC cathode coatings (**Figures 2 and 3**), were assembled using 2 L glass beakers (Pyrex, model 1000; ~ 16.0 cm height × 13.5 cm diameter) filled with wastewater (described below). Each MFC contained an air cathode that floated on the wastewater surface, an anode positioned parallel to and 6 cm below the cathode, and an Ag/AgCl reference electrode (BASi, −0.397 V *vs.* standard hydrogen electrode, SHE) positioned at the edge of the beaker approximately 2 cm beneath the wastewater surface (**Figure 2**). These batch reactors were not stirred (*i.e.*, mass transport is dependent on convection and diffusion) and operated in parallel using a multichannel potentiostat (Solartron 1470E, Ametek) under the control of software (Multistat, Scribner Associates) at room temperature under a chemical hood for odor control. DI water was periodically added to compensate for evaporation.

Wastewater was collected from a large WWTP on the US east coast. Samples were taken from the center of the primary clarifier and the primary settled solids tank and stored in plastic 1-gallon jugs at 4°C. The MFC experiments were started within two days of sampling, thus reducing the potential effects of storage.

A preliminary study (results not shown) indicated that the organic content of the primary clarifier effluent (COD of ~ 150 mg/L) was rapidly depleted under batch conditions. To maximize the anodic biofilm growth and lengthen each batch cycle, additional organic matter in the form of primary solids was added to the primary clarifier wastewater at a 1:4 ratio (COD of ~ 1500 mg/L). Organic matter was replenished at the completion of each batch cycle (after 3–4 weeks) by adding 200–300 mL of primary clarifier influent or a primary influent–primary solids mixture. The ratios of primary influent-to-primary solids were selected based on the performance of the MFC during the previous cycle to maximize power production.

2.4 Data Collection and Analysis

The general electrochemical treatment of the 6-MFC stacks was as follows: 1) Each of the six MFCs was placed in the open-circuit mode for 48 h; 2) subsequently, the MFCs were operated in three-electrode configuration with the working electrode (anode) at −0.200 V *vs.* Ag/AgCl and the cathode serving as the counter electrode. Poising the anode at this low potential selected for anode-respiring bacteria (ARB), resulting in production of an electrical current; 3) after the current had stabilized, each MFC was placed in the open-circuit mode for 24 h; 4) a polarization analysis was performed in the two-electrode configuration using the potentiostat by sweeping the cell voltage (potential of the cathode *vs.* potential of the anode) from the open-circuit voltage to the short-circuit voltage at 1mV/s (the cathode was connected to the working electrode input, whereas the anode was connected to the reference- and counter-electrode inputs); 5) each MFC was placed in the open-circuit mode for 3 h; 6) finally, each MFC was discharged for 3–14 days at the cell voltage that resulted in the highest power, as determined using the polarization curves (generated from the two-electrode configuration).

Cyclic voltammograms of the anode were periodically recorded on day four, ten, and eighteen after the 48 hour startup period for the MFC stack. The MFCs were temporarily switched back to the three-electrode configuration and electrode potentials *vs.* the Ag/AgCl reference electrode were measured periodically using a hand-held multimeter (Fluke, mode 179). A MFC was operated in the three-electrode

configuration to approximate the condition in which the cathode was non-limiting, allowing the anode to be set at a known potential when growing an ARB biofilm on its surface. The data generated from these MFCs determined whether the anode or cathode was limiting the performance of the MFC operating in the three- *vs.* two-electrode configuration ("fuel-cell" cell mode).

Current recorded in the three-electrode configuration was normalized to the anode surface area. Current and power recorded in the two-electrode configuration were normalized to the cathode geometric surface area based on reports that the oxygen reduction reaction at the cathode is often the limiting reaction in an MFC (Ahn and Logan 2010, Rismani-Yazdi et al. 2008). Due to the small sample size, data for each replicate are presented.

2.5 Determination of Mn Leaching from Cathodes

Based on the initial loading of the cathodes (0.5 mg 20% w/w MnO_2/VC per cm^2 cathode surface area), it was determined that each cathode (SA = 21 cm^2) contained 2.41 ± 10^{-5} moles Mn or 1.33 mg. Wastewater samples (200 mL) were analyzed at the start of the experiment and after 17 days to measure the change, if any, in total Mn concentration using EPA Method 200.8 (E.P.A. 1994). The initial concentration of manganese in wastewater was 0.38 mg/L.

Results and Discussion

3.1 Three-Electrode Configuration of MFCs

The current density of the MFCs recorded during the initial poising of the anode potential in the three-electrode configuration at -0.200 V *vs.* Ag/AgCl was performed in diluted primary sludge wastewater (1:4 primary settled solids: primary influent). The onset and subsequent increase in current indicated that anode was colonized by ARB. This colonization resulted in the oxidation of organic matter at the anode (**Figure 4**). The plateau in current density most likely indicated a mature catalytic anode

biofilm (Strycharz-Glaven and Tender 2012). Possible variability in the anode surface area at the microscopic level and variations in the wastewater composition may have contributed to the variation in current and power values among the replicates (Table 1). Moreover, the maximum current density with this MFC configuration, assuming a non-limiting cathode, was 250 to 500 mA/m^2 anode geometric surface area.

The current density of the MFCs decreased for all reactors during the second period when the anodes were poised at -0.200 V *vs.* Ag/AgCl (**Figure 4**). During this period, the current densities of the Mn-coated electrodes exceeded those of the Pt-coated electrodes. In particular, one of the Mn-coated MFCs showed current densities of up to 330 mA/m^2, compared to 100–120 mA/m^2 for MFCs with Pt-coated electrodes. From days 5 to 13.75, polarization analyses were performed on the MFCs (data not shown) prior to their discharge into a two-electrode configuration (**Figure 5**).

From day 13.75 to 14.75, the MFCs were placed in the open-circuit mode and 200 mL of the primary solids-influent mixture was added to compensate for any depletion of organic matter. At days 15–19 (Figure 4), the MFCs were unable to attain the original current densities in the three-electrode configuration, with the exception of one reactor (Mn3). These data suggest that the fuel cells were anode limited relative to the first period of operation in the three-electrode configuration. Presumably, some change in the anodes or operating conditions (pH, DO levels, and temperature remained constant) caused the decline in current due to insufficient replenishment of the organic matter depleted by the polarization analysis.

From days 28 to 32, the current density was affected by the addition of primary effluent and the COD was markedly lower than that of the original mixture (COD of ~ 150 *vs.* ~ 1500 mg/L). As described above, polarization analyses were performed on the MFCs (**Figure 6**), and they were placed in the open-circuit mode during the intervening period. The current densities did not reach the values at days 0–5, which is consistent with an organic-matter-limited current.

From days 47–50, the MFCs showed current densities comparable to those at days 0–5 after the addition of 300 mL of a 1:1 mixture of primary settled solids to primary influent (COD of ~ 2850 mg/L). As above, polarization analyses were performed on the MFCs (data not shown), which were next discharged in two-electrode configuration and in the open-circuit mode during the intervening period. Here, addition of wastewater with a high organic matter content enabled the MFCs to overcome the organic matter limitation that occurred in the previous two batch cycles. Steady-state current densities of slightly more than 300 mA/m^2 anode geometric surface area were observed at days 47 to 50.

3.2 Polarization and Maximum Power Densities

Initial polarization tests on day 9 indicated that MFCs with manganese-based cathodes produced higher maximum power densities at higher current densities than those with platinum-based cathodes (**Figure 6**). The average maximum power of the manganese MFCs was 40.1 ± 3.7 mW/m^2 (normalized to cathode surface area), compared to 30.1 ± 0.5 mW/m^2 for the platinum MFCs. Previous tests with catalyst-free air cathodes indicated that the addition of platinum or manganese increased the maximum power of the MFC by at least fourfold (data not shown).

The polarization tests performed on days 37 and 55 after addition of new wastewater revealed a shift in the maximum power production between MFCs with platinum- and manganese-based cathodes. Those with platinum cathodes eventually achieved higher maximum power densities at higher current densities than the MFCs with MnOx cathodes. However, both the average maximum power densities and the current densities increased as the number of MFC cycles increased. This may reflect how the catalyst responded to the addition of wastewater with a high organic matter content, particularly at days 47–50. While these values increased over time, their variability also increased, and the maximum power density values for Mn-cathode MFCs at 9 days were within one standard deviation of those at 55 days (Figure 6).

3.3 Cyclic Voltammetry

Cyclic voltammetry (CV) was performed periodically on the anode of each MFC to evaluate the development of the catalytically active ARB biofilm (Figure 7). No significant differences in anode voltammograms were observed between the two types of coating used in the cathodes of the MFCs. All CV tests performed on the anodes with established biofilms showed half-wave oxidation potentials of −0.35 V *vs.* Ag/AgCl. This function has been reported by others and showed that the anodes were colonized by ARB (Kubannek et al. 2018, Lee 2018, Suzuki et al. 2018). As indicated in Figure 7, the highest current densities were detected after the initial growth in the three-electrode configuration. After 6 days of MFC operation (two-electrode configuration), there was a substantial decrease in current, likely due to depletion of organic matter. After replenishment of organic matter by addition of primary clarifier wastewater and settled solids, the current density increased. However, most MFCs did not recover to their initial maximum current densities, which is consistent with the initial operation in the two-electrode configuration.

3.4 Power Density of MFCs

The results obtained when the MFCs were discharged in two-electrode configuration showed that during polarization the MnOx or platinum coated cathodes were at cell voltages of 0.5 and 0.45 V, respectively (*vs.* Ag/AgCl reference) from days 6–13. All MFCs were at a cell voltage of 0.4 V during the subsequent discharges (days 20–25 and 35–45). The power density over time (**Figure 5**) indicated marked variability in power production by the MFCs. After approximately 1 week of operation, the MFCs experienced declines in power density, likely due to depletion of available organic matter. Additionally, leaching of Mn(II) from the MnO_X-coated cathodes (Section 3.5) may have contributed to the loss of power density of MFCs with manganese-based cathodes. Moreover, the MFCs with platinum-based cathodes produced higher average power densities than did those with MnOx over time. This is consistent with the suspected limitations of polarization analysis in MFCs with respect to longer-term power generation at a fixed cell voltage. It is also consistent with a previous study that compared Pt and MnO_X-coated cathodes in a two-chamber MFC, in which Pt MFCs achieved maximum power densities of 190 mW/m^2, compared to

160 mW/m^2 for Mn MFCs (Roche and Scott 2009). The higher power densities from our MFCs may be due to differences in the substrates and configurations. Regardless, all MFCs achieved significantly lower raw currents (non-normalized), when discharged in the two-electrode than the three-electrode configuration. This is consistent with a cathode-limited current and power density when discharged in two-electrode configuration.

3.5 Electrode Potentials

Cathode potentials under open-circuit conditions showed that the platinum-coated cathodes had higher OCPs after 14 days of operation (Table 1). Additionally, the manganese cathode potentials decreased over time, while platinum cathode potentials returned to their original values after 31 days of operation with wastewater. This change could be due to the solubilization of manganese based on the measurements of the total Mn(II) present in the wastewater before and after the cathodes were introduced. A study in acetate medium and a two-chamber configuration suggested that the MnO_X loading density is strongly associated with the performance of MFCs the current density increased 100% with a 5x higher loading on the electrode (Roche et al. 2010). After 17 days of operation with addition of only deionized water, Pt-MFC anolyte showed no change in total Pt levels, while the Mn-MFC anolyte exhibited Mn(II) concentrations ranging from 1–1.9 mg/L. The mass balance of total Mn in the system using the initial concentration in the wastewater and the 0.5 mg/cm^2 of 20% w/w MnO_X/VC loading of the cathode indicated that more than 90% of Mn was lost from the cathode surface over the duration of the experiment. While the gradual decrease in Mn-coated cathode potentials could indicate loss of the catalyst over time, the cathodes maintained higher potentials than a plain carbon cloth cathode (data not shown) since they were operated in batch mode. The leaching of metals into wastewater streams is of significant concern however there are several strategies that can developed around improvements in the cathode design or contained with new developing research fields like “solvometallurgy” (Binnemans and Jones 2017).

Platinum nanoparticles are an established ORR catalyst but are too expensive and not stable in unpurified waste streams(Sawant et al. 2017) . Thus, the use of MnOx for wastewater applications may have two outcomes. Generally, the redox couple associated with the biomineralization of Mn(II) to MnO_2 has a lower formal redox potential (+0.360 V *vs.* saturated calomel electrode, SCE) (Rhoads et al. 2005) compared to oxygen reduction (1.223 V *vs.* SCE) under the same conditions. Although the rate of reduction of Mn(IV) to Mn(II) is higher than that of oxygen reduction, the amount of current it typically creates is greater based on kinetics. This delicate balance between the rate of reduction and the thermodynamic driving force can be exacerbated in MFCs, as the current produced is so low that a cathodic catalyst with a greater thermodynamic driving force can produce current comparable to a catalyst with a considerably lower potential driving force. Thus, the ultimate fate of manganese reduction under these conditions may be limited by diffusion from the surface not by maintaining an active ORR heterogeneous catalyst for oxygen reduction. This balance is an advantage for a MFC operated in batch mode but severely limits its application in a continuous flow-through system. However, this can be overcome by reducing the rate of diffusion using manganese-tolerant microorganisms that catalyze the biomineralization of manganese (Yang et al. 2013) or testing conductive polymer supports like polyaniline in order to immobilize these catalysts as the fuel cell is operated (Li et al. 2018).

Our study focused on the impact of MnOx catalysts on the performance of MFCs compared to platinum electrodes but research into the ability of cathodic biofilms to catalyze oxygen reduction is increasing in frequency and intensity and could aid in the cycling of metal catalysts on the cathode surface (Liu et al. 2018, Milner and Yu 2018). Such biofilms could catalyze oxygen reduction directly by producing enzymes or indirectly through the reductive precipitation of manganese oxides (Erable et al. 2012). In some cases, these biofilms have been deliberately enriched by leaving the system in the open-circuit mode or at high (> 1000 Ω) external resistances (De Schamphelaire et al. 2010, Faimali et al. 2008, Song and Jiang 2011). In other cases, the growth of cathodic biofilms has been promoted by poising the cathode at a sufficiently low potential (Chen et al. 2010, Liang et al. 2009). Cathodic biofilms utilizing the oxygen

reduction reaction may have developed during open-circuit periods or been promoted by the presence of Mn species around the cathode. This biological activity, while not explored in this study, may explain the continued strong performance of MnO_X-coated cathodes after substantial loss of catalyst.

Conclusions

Our results showed that MFCs can be configured to oxidize the organic matter present in domestic wastewater, while producing a considerable power output from a primary settling tank. The anaerobic ARB in the MFCs minimizes the need for costly secondary aeration treatment. Secondary aeration treatment is applied in the majority of WWTPs across the US and western hemisphere and has a significant energy input requirement for the oxygen supplied during aeration processes. Energy production, which was the purpose of using MFCs in wastewater treatment, is a function of the organic matter content and cell voltage.

This research addressed the feasibility of using MnO_x as a low-cost alternative cathode catalyst to platinum (Pt). Our results show that cathodes coated with MnO_x and Pt performed similarly in wastewater. While manganese oxides are less costly than platinum, their use is limited by the rapid dissolution of Mn^{2+} from the cathode surface. Following its solubilization, Mn leached from the cathode surface and could no longer function as an ORR catalyst. This dissolution may be thwarted by use of higher concentrations of ion-binders, recycling using manganese-tolerant microorganisms, or higher current output from the MFC.

The open structure of the air cathode MFC design investigated in this study showed promise for use in wastewater treatment. Because batch reactors were used in this study, the reactions were largely substrate limited, and the power densities would improve under continuous-flow conditions. Additionally, increasing the electrode surface area-to-volume ratio of the reactor would aid in determining whether MFCs are capable of removing considerable quantities of COD at typical WWTP retention times.

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Chapter 3

Preliminary bench-scale investigation of DC Water process streams as microbial fuel cell feedstocks

Aaron Leininger[1], Mark Ramirez[2], Birthe Kjellerup[1]

[1] University of Maryland, College Park Department of Civil and Environmental Engineering

[2] DC Water Blue Plains, Resource Recovery, Washington DC

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Abstract

As a first step towards developing a scaled-up microbial fuel cell installation, preliminary testing of microbial fuel cell feedstock blends was conducted in 300 mL batch reactors using primary effluent, filtrate, and 60:40 and 80:20 (primary:filtrate) blends. Microbial fuel cell cathodes were plain carbon felt and did not have an applied chemical catalyst. Relative performance was gauged by removal of chemical oxygen demand, volumetric energy recovery, and coulombic efficiency. In general, higher strength feedstock blends resulted in higher volumetric energy recovery and chemical oxygen demand removal but lower coulombic efficiency. Highest volumetric energy recovery was observed to be 0.0023 kWh / m^3 of wastewater in the 60:40 blend. As a result of these findings, a 60:40 blend was proposed as a feedstock for the pilot system.

Materials and Methods

Experiments were conducted in modified 300mL commercially available educational MFC kits (Mudwatt, USA). The anode and cathode electrode were held separate at a distance of 3cm by a plastic separator and the reactor was filled with wastewater blend of primary effluent, filtrate, and 60:40 and 80:20 (primary:filtrate) blends. Reactors were operated in triplicate, with two reactors of each blend in closed-circuit mode and a third in open-circuit operation. Initially, the reactors were operated in closed circuit mode across a 1000Ω resistor. At the end of 41 days of operation, the external resistance was lowered to 100Ω. Voltages across external resistors were recorded using a digital multimeter equipped with a switching card (Keithley 2700, Keithley Instruments, USA) using KickStart software (Textronix, USA) every five minutes. Current was calculated using Ohm's law and normalized by cathode projected surface area (Acat = 0.00567 m^2). Determination of chemical oxygen demand was conducted in triplicate from each reactor using an established method (COD TNT Plus, Hach, USA). Coulombic efficiencies and volumetric energy recoveries were calculated using a trapezoidal integration method of current using the established method (Logan et al. 2006).

Reactors were refilled every 3-4 days with fresh wastewater collected the same day from DC Water's Blue Plains facility. Primary effluent was collected from the effluent channel of a primary clarifier. Filtrate was collected from the filtrate well in the basement of the final dewatering building

Discussion

The results reported here were the second attempt of this experiment. During the first attempt, filtrate was initially collected directly from the effluent line of a belt filter press. During one fill cycle, the current production of filtrate containing MFCs dropped to near zero (insert value, or percent decrease) and did not recover in subsequent fill cycles of fresh substrate. After this, the sampling location was changed to a composite sample from the filtrate well located in the basement of the final dewatering building. After

this change, electrodes were cleaned in a series of bleach, hydrochloric acid, and sodium hydroxide rinses and the experiment was repeated. These results are the data reported here. It is considered likely that the crash in current production of filtrate-containing MFCs could be attributed to the cleaning of the belt filter presses with bleach and detergents before collection of samples. Following change of the filtrate source, no subsequent crashes in current production were observed. This observation suggests that microbial fuel cell performance may be adversely affected by disturbances or disruptions in feedstock characteristics.

During the initial several fill cycles in startup, the data were grouped closely between replicates (Figure 1). However, in later tests during the operational phase, operational differences became apparent between duplicated closed-circuit reactors. For example, average and peak current densities in closed circuit filtrate MFCs varied by a factor greater than two in the first fill cycle shown in the operational phase (Figure 2) despite receiving the same feedstock blend. This could be attributed to divergent microbial growth, but characterization of microbial communities was not conducted in this preliminary work.

Benefit of microbial fuel cell implementation to wastewater treatment can occur through direct energy recovery and treatment of wastewater. While operated at the same batch length, filtrate reactors removed over twice the COD of primary effluent reactors (602 vs 138 mg/L). A positive relationship was observed between wastewater influent COD and COD mass removal, while an inverse relationship was observed between wastewater influent COD and COD percent removal. Coulombic efficiency was observed to be higher in low-strength wastewaters than in high strength blends. This measure indicates that a greater proportion of organic oxidation was associated with electron transfer to electrodes in lower strength wastewaters. This could be a result of mass transfer limitations or kinetically limiting reactions at the electrodes when it is considered that the difference in COD removal between different blends is much more significant than the difference between current densities between different blends. Additionally, filtrate contained more suspended flocs than primary effluent which may have contributed to a higher proportion of microbial growth and activity in the suspended rather than attached mode.

It is noteworthy that in this work high strength wastewaters and blends containing high strength wastewaters performed equally or better to primary effluent. Filtrate contains around 1500 mg/L total ammonia nitrogen at a pH near 8, suggesting significant speciation as free ammonia nitrogen. Several studies (Hiegemann et al., 2018; Kim et al., 2011; Nam et al., 2010) have reported inhibition of microbial fuel cell function at total and free ammonia nitrogen levels near or below these levels. However, those studies did not test the long-term inhibitory effects of ammonia nitrogen using MFCs fed high strength dewatering wastewater over an extended time and therefore did not study the ability of electroactive biofilms to adapt to high ammonia environments. An ordination of biofilm samples from a previous study suggested divergence of anodic communities because community structure was shown to be more dependent on initial wastewater type than whether the sample was an initial or final wastewater or anode biofilm (Appendix A). This work suggests that electroactive biofilms grown over time in high ammonia environments may function differently or have different community structure than those grown in primary effluent or in buffered media.

Conclusion

The preliminary work suggested that the addition of high strength dewatering wastewater to the main process stream (primary effluent) may improve the ability of an integrated MFC system to recover energy and degrade organics. At DC Water Blue Plains, flows of primary effluent are much higher than filtrate (270 vs 1.2 MGD). However, organics are around ten times as concentrated in filtrate. Side stream treatment of high strength wastewaters may be an effective application of bioelectrochemical techniques at wastewater treatment plants. As a result of this work, a feedstock blend of 60:40 (primary effluent : filtrate) was proposed for the pilot reactor study.

Chapter 4

Evaluation of microbial fuel cell application to wastewater process streams by pilot-scale system net energy balance

Aaron Leininger[1], Matthew Yates[2], Mark Ramirez[3], Birthe Kjellerup[1]

[1] University of Maryland, College Park Department of Civil and Environmental Engineering

[2] Center for Bio/Molecular Science and Engineering, Naval Research Laboratory, Washington, DC

[3] DC Water Blue Plains, Resource Recovery, Washington DC

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Abstract

A novel modular microbial fuel cell system was developed and tested over an extended period for energy recovery and removal of chemical oxygen demand in an 800L flow-through system with a blend of high-strength real domestic wastewater. The system consisted of five modules with bottle-brush anodes and cathodes with no treatment or chemical catalysts. This is the first study to assess both the electrochemical behavior and spatial differences in biofilm communities of large electrodes in a wastewater MFC. Over a 43-day operational study period, removal of total chemical oxygen demand averaged 15%, or 110 $□_{□□□□}/(□^3 * □□□)$. Energy recovery was low in comparison to other pilot MFC systems, but the net energy benefit of this system compares favorably to some other system architectures.

Introduction

Microbial fuel cells (MFCs) are bioelectrochemical systems which have promise as an alternative unit process to the activated sludge process for wastewater treatment because of their ability to oxidize and remove organic materials from wastewater without the need for mechanical aeration. Wastewater treatment plants are the largest point-source user of electricity in most municipalities, and the activated sludge process is the largest user of energy within a wastewater treatment plant (Menendez, 2010). In addition to being energetically costly, the activated sludge system produces large amounts of waste sludge which must be managed and disposed of at cost. MFCs use electrodes in two spatially separated redox zones to allow biofilms in anaerobic zones to "breath" oxygen that is present at another location. In doing so, biodegradation of organic matter is enhanced by providing a favorable electron acceptor and energy can be recovered, although the value of this energy is typically vastly outweighed by the value of treatment energy savings.

The behavior of MFCs has been widely characterized in laboratory settings and often purported to have great potential benefit to wastewater treatment, but only a handful of studies have examined scaled-up systems using real domestic wastewater under realistic operational settings (Feng et al., 2014; Hiegemann et al., 2016; Zhang et al., 2013). All of these studies have used platinum catalysts at the cathode to facilitate the rate-limiting step of the oxygen reduction reaction. Platinum is too expensive to be utilized at a large scale in domestic wastewater treatment. Because of this, biocathodes have been proposed as an alternative, where a biofilm community exists which uses the cathode as an electron donor (Du et al., 2014; Jiang et al., 2017; Milner et al., 2016). However, the microbial community structure of biofilms existing on cathodes in scaled-up wastewater MFCs has not been characterized in the literature. In addition, large electrodes in scaled-up MFCs may have biofilm communities that vary spatially.

This study aims to better understand the processes and mechanisms that occur in a scaled-up wastewater MFC in order to evaluate their potential as an alternative unit process and inform future design. This study reported here is one of the largest installations reported in the literature, utilizes two process streams

and a novel modular design using brush anodes and brush biocathodes, and will be the first study to analyze biofilm community structure and dynamics in a scaled-up wastewater installation.

Materials and Methods

The pilot MFC system consisted of 5 modules inserted into an 800 L rectangular fiberglass tank (Formed Fiberglass, Pennsylvania, USA). The tank was based on a 10% dimensional scale of an existing secondary reactor/aeration basin at DC Water (5.5'x3.5'1.5'). Evenly-spaced modules alternated and stretched 2/3^{rds} of the way across the tank to function as baffles and promote a plug-flow pattern. Modules were numbered according to their location in the flowpath, with module 1 nearest to the inlet and module 5 closest to the tank outlet. Modules were supported in place in the reactor by an overhanging PVC support structure. The system was located in a climate-controlled shipping container located in a parking lot.

Modules were constructed from PVC to support anodes and cathodes. Both anode and cathode were carbon fiber brushes 3" in diameter and 28" long with titanium center wire (Mill-Rose Company, Ohio, USA). Brushes were untreated by any means upon delivery. Three brushes were fixed with cable ties to the top of the module with the centerline at water level and six brushes were attached to the frame in a V-shape to form an anode assembly. Insulated copper wire was attached to electrodes and sealed with electrical insulating resin (Scotchcast 2131, 3M, Texas, USA) using a 15mL centrifuge tube as a mold. Contact resistances from far end of brush to end of wire lead averaged $1.5 \pm 0.7\Omega$. Wire leads from anode and cathode brushes were combined and connected across the external load such that each module consisted of a single cell. Voltages across external resistors were recorded with a digital multimeter (Keithley 2700, Ohio, USA) at 5 minute intervals. An Ag/AgCl reference electrode (Gamry Instruments, Pennsylvania, USA) was mounted on the outside of module 5 and used to log cathode potentials.

Unamended primary effluent and a belt-filter press dewatering wastewater (filtrate) were fed to the reactor via peristaltic pumps at a 60:40 (primary effluent: filtrate) ratio with a hydraulic retention time of

1 day. Primary effluent was pumped 200 yards from the effluent channel of a clarifier to a 1000L tote equipped with an overflow line via a 0.33 HP submersible pump (SSM331PC-1, Myers, Ohio, USA). From the tote, primary effluent was fed into the reactor via a peristaltic pump (Masterflex 7553-30, Cole-Parmer, Illinois, USA) at a rate of 480 L/day. Filtrate was fed directly from an open-air feed tank to the MFC reactor via a large peristaltic pump (74204-18, Cole-Parmer, Illinois, USA) at a rate of 320 L/day. Influent was introduced in the corner of the tank adjacent to the first module in the flowpath and most distant from the effluent weir to promote a plug-flow system.

Cyclic voltammagrams of anode and cathode arrays from each module were recorded at various stages of the reactor operation. A counter electrode was constructed of a lattice of 6 electrode brushes identical to those used in module construction and placed 25 cm from the modules. Scans were run with a potentiostat (Reference 600, Gamry Instruments, Pennsylvania, USA) starting at 0.35 and scanning from 0.4 to -0.75 V vs Ag/AgCl at a scan rate of 1 mV/s. Power curves were recorded by varying the external resistance in a stepwise pattern from 1000Ω to 1Ω every 30 minutes.

Operation of the system began with a single module (module 1) placed in the tank in batch reactor mode in late July 2018 in order to assess the viability of the reactor design and construction. Reactor was filled with 60:40 wastewater blend at roughly weekly intervals.

After the batch system, four additional modules were placed into the tank and continuous flow operation began in September 2018. Background CVs were run on all modules upon placing in the tank on September 19th. The modules were placed in open circuit mode on September 19th and open circuit potentials were monitored for 14 days from September 24th to October 8th. The electrodes were then connected across a 1000Ω resistor for 38 days until November 16th. On November 16th, a second round of cyclic voltammetry was conducted on all electrodes. From November 16th to the present, all modules have been connected across a 15Ω external resistance.

Influent and effluent samples were taken on a regular basis and analyzed for soluble and total chemical oxygen demand within 24 hours using triplicate tests and a standard method (Hach TNT HR). Soluble samples were first filtered using a 0.45um syringe filter. Aliquots of influent and effluent have been archived in a freezer and at the time of writing are being analyzed for total Kjeldahl nitrogen using a standard method (Hach TNT s-TKN). At the time of writing, an automated multiprobe (pH, conductivity, and temperature) (ThermoScientific AquaPro) is being installed with probes at the entrance and exit to the reactor to determine what relationship these parameters have on MFC performance.

Biofilm Analysis

Biofilm samples were taken from different locations along the length of anode and cathode modules at different stages of reactor operation. Brush fibers with attached biofilm from the full area of brush cross section were taken with flame sterilized scissors and placed in 15mL centrifuge tubes for later analysis. Three samples were taken from each electrode array, one at the end closest to wire attachment, one midway across the brush, and one at the distant end of the brush, for a total of 6 samples per module per sampling. Biofilm sampling occurred at the end of batch reactor operation from Module 1, at the end of open circuit operation from all modules on October 8th, and before lowering the external resistance from 1000Ω to 15Ω on November 16th. Biofilm will also be sampled on during the week of December 17th, after 1 month of operation with lowered external resistance. At this time, cathode biofilm samples will be obtained from three locations: above water, at water-air interface, and from brush fibers below the surface.

DNA will be extracted from biofilm samples using a commercially available kit (PowerSoil DNA Isolation Kit, MoBio) and Illumina sequencing of the V3-V4 region of the 16s rRNA gene will be performed. Data will be processed through the DADA2 pipeline to determine relative abundance of taxa and perform alpha and beta diversity analyses.

Energetic Calculations

Loading of COD (kg/day) was determined as the product of flow rate (Appendix) and influent/effluent COD concentrations. Average COD removal over the study period was determined by the difference between trapezoidal integral approximations of the COD load in and load out. Maximum volumetric energy recovery (kWh/m^3) was determined by EQ-1 during the period of highest power. Coulombic efficiency will be calculated using the sum of the current from all modules and COD removal over the study period.

EQ-1 $$\square\square\square_{\square}\left(\frac{\square\square h}{\square^{3}}\right) = \sum(\square\square\square\square\square\ \square\square\square\square\ \square\square\square\ \square\square\square\square\square\square\square\ (\square\square)\ \frac{24\, h\, \square\square\square\square}{0.8\ \square^{3}}$$
*

Results and Discussion

Start up

The system was initially operated for 18 days in the flow-through tank with all modules in open-circuit mode. Open circuit cell voltages (OCVs) and the cathode potential of module 5 were recorded during this time (Figx). The highest observed OCV was 0.193V in module 2. This is significantly lower than an observed OCV of around 0.7V in a flat-panel wastewater MFC with Pt-catalyzed cathodes (Feng et al., 2014). The observed cathode potentials during this period (-.485 to -.428 V vs Ag/AgCl) were lower than values reported in WW MFCs of other architectures, such as a flat cloth cathode with activated carbon catalyst in brewery wastewater (0.025- .1 V vs Ag/AgCl) (Dong et al., 2015). Between days 8 and 14, large peaks in OCV occurred on a daily cycle. This may be attributed to diurnal temperature swings, but temperature probes were not present in the system at this time and this cannot be validated.

Treatment and Energy Performance

Throughout operation in closed-circuit mode, the MFC system averaged a current output of 0.26mA. However, after the first day total current output varied from 0.09 to 0.7 mA. Current production in an operational MFC can vary by biofilm development, growth or sloughing, (Babauta et al., 2014; Beecroft et al., 2012; Sun et al., 2017), or changes in pH (Jadhav and Ghangrekar, 2009) temperature (Ahn and Logan, 2010), chemical oxygen demand (Di Lorenzo et al., 2009), ionic strength (Liu et al., 2005), hydraulic retention time (Walter et al., 2016), or ammonia concentrations (Hiegemann et al., 2018). In a pilot scale system, all of these parameters are in constant flux affected by variations in constituent wastewater characteristics and variations in flow rate into the reactor (Figure 3).

The study reactor was fed two wastewaters with different characteristics. Filtrate has a higher average sCOD concentration (1567±11 vs 122±9 mg/L) conductivity (10.11 mS vs 828 μS), pH (around 8 vs around 7) temperature (N.D) and total ammonia nitrogen (1892±43 vs 30.3±1 mg/L) than primary effluent. Because of this, variations in the flow rate into the reactor could have significant impacts on the system performance. For example, during the 6 day period (Table 1, days 21-27) when filtrate flow stopped because of a mechanical issue, COD loads in and out dropped (Figure 4), the average conductivity, pH, and ammonia decreased and the hydraulic retention time increased. During this period, the total current production dropped from near 0.7 mA to less than 0.2 mA. In another pilot-scale MFC system, a "shock load" of high-ammonia solids-dewatering wastewater was shown to crash the current production of a system acclimated to primary effluent (Hiegemann et al., 2016). In this study, the absence of a high-ammonia solids-dewatering wastewater was shown to decrease the current production of a system acclimated to containing it. This suggests that given time, MFC electrode biofilms can adapt to different feedstocks.

While the system described in this study compares favorably with other studies in its COD load removal (Table 1) (Feng et al., 2014; Hiegemann et al., 2016), it compares unfavorably with other systems described in the literature in its ability to harvest energy from wastewater. The volumetric energy recovery (NER) of 6.21 x 10^-6 kWh/m^3 reported here is three orders of magnitude smaller than the next largest value from reported pilot-scale wastewater MFCs (Table 1). This may be partially attributed to the absence of platinum catalysts on the cathode on this system and poor cathode performance. Module 5 cathode half-cell potentials measured from this system during polarization tests ranged from -0.475 to -0.449 V vs Ag/AgCl.

The low cathode potentials may be explained by the size and orientation of the cathode electrode brush. This is the first study to analyze brush cathodes in a scaled-up wastewater MFC, and there is no existing knowledge of the bioelectrochemical behavior of biofilms and electrodes on these larger electrodes. In this system, the electrode and biofilm spans at least three distinct zones: open air brush fibers (0-3.81cm above water), brush fibers at water surface, and fibers below wastewater surface (0-3.81cm below air-water interface). This differs from other scaled-up wastewater MFCs which use flat cathodes (Dong et al., 2015; Feng et al., 2014; Hiegemann et al., 2018). A single small electrode placed in any of these three areas would measure its own individual (different) redox potential. When combined as an array of three large brushes, each spanning different zones, the cathode array arrives at a single half cell potential, where the aerobic portions of the brush may serve to buoy the potential up and the deeply submerged region may drag the potential down. It is possible that distinct biofilm communities inhabit the different regions of the cathode and exchange electrons with each other via the brush fibers. For example, it may be more favorable for an oxygen-reduction reaction catalyzing biofilm at the air-water interface to accept electrons from a heterotrophic biofilm on a submerged region of the cathode than it would be to accept electrons via the external circuit/resistance from the anode. In this way, the large cathode array may function more like a “snorkel” (Erable et al., 2011; Viggi et al., 2017) with an internal exchange of electrons through the electrode than as a distinct cathode in an MFC.

The authors will conduct sequencing of biofilm communities in the different spatial regions of the cathode arrays in order to test this hypothesis. Additionally, the authors plan to test the effect of submerged or "deep" cathode brush fibers on cathode potential and module power recovery by cutting the submerged brush fibers off of one of the modules and comparing the power of this module with other modules in the system and its performance before cutting off submerged fibers. It is hypothesized that this removal of submerged fibers will result in an increase in cathode potential and module power production.

Cyclic voltammetry was used as a tool to describe the electrochemical behavior of MFC electrodes throughout the study. In large electrodes with complex analytes, CV is a qualitative tool, but can provide information about the processes of biofilms occurring at electrodes by comparing initial background (no biofilm) to acclimated biofilm-covered electrodes. After 36 days of operation in closed-circuit mode, the CVs of both anodes and cathodes changed. The current response from the anode increased significantly at higher applied potentials greater than -200mV vs Ag/AgCl, but is not clearly higher in the region where it operates during MFC function (-400 to -500 mV vs Ag/AgCl). The high current yield from the anode (up to 600mA) suggests that a high rate of reaction and possibly large organic removal could be attained in a system where the anode arrays are potentiostatically controlled and connected to a current sink. This current yield is based on a single scan rate however, and a scan-rate analysis would be needed to determine the sustainable current yield. The CV from the cathode may reveal a more important process. The initial cathode CV showed a large current-limited region from -350 to 450 mV vs Ag/AgCl where current was near-zero. However, after biofilm growth, the cathode exhibits an anodic behavior in this region with catalytic currents exceeding 200mA. This supports the hypothesis that the cathode may develop "anodic regions" where biofilms in submerged regions may use the brush fibers as an electron acceptor.

Conclusions

The modular MFC system is a promising method of attaining low-energy, low-cost wastewater treatment. However, the system as presented in this study is not capable of entirely replacing the activated sludge system in wastewater treatment, but rather serving to augment the treatment process train. This study suggests that MFCs may be suitable to side-stream treatment of high strength dewatering wastewaters, where they may have an outsized energetic benefit. Furthermore, microbial transformations and biofilm community structure, dynamics, and spatial distribution was described in a large-scale wastewater system for the first time.

Chapter 5

Evaluation of microbial electrolysis cell application to wastewater process streams by liter-scale single-chamber systems

Aaron Leininger[1], Enna Wu[1] , Matthew Yates[2], Birthe Kjellerup[1]

[1] University of Maryland, College Park Department of Civil and Environmental Engineering

[2] Center for Bio/Molecular Science and Engineering, Naval Research Laboratory, Washington, DC

[3] DC Water Blue Plains, Resource Recovery, Washington DC

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Abstract

A meso-scale testing platform for microbial electrolysis cells was developed and used to evaluate wastewater process streams for treatment energy saving and biogas production potential. Solids dewatering filtrate and a 60:40 blend (primary effluent:filtrate) produced similar sustained current densities of around 30 □□/□², while primary effluent current densities were less than 10 □□/□². Qualitative gas analysis of produced biogas showed methane yield with all feedstocks, but hydrogen yield in only positive control, suggesting the utilization of hydrogen by microbes. Closed circuit systems removed significantly chemical oxygen demand than open circuit replicates in high-strength wastewater systems, up to 283 ± 117 mg/L in a one-week batch cycle. This work suggests that microbial electrolysis cells may be an effective unit process in wastewater treatment for energy recovery and for the treatment of recalcitrant organics in high-strength wastewater process streams.

Introduction

Microbial electrolysis cells (MECs) are a bioelectrochemical system which use an anode-respiring biofilm to oxidize materials on one electrode and reduce another chemical, often protons, on another electrode. The reaction is driven by a supplementary voltage applied either by a potentiostats or a power source. MECs are a promising technology as an alternative to the conventional activated sludge process used in most wastewater treatment plants because of their ability to oxidize wastewater organics without the need for costly aeration and simultaneously recover usable energy in the form of biogas (Escapa et al., 2016).

MECs have been tested for various environmental engineering applications, including enhanced anaerobic digestion (Asztalos and Kim, 2015), in-situ production of peroxide for wastewater disinfection (Sim et al., 2018), bioremediation of heavy metals in industrial wastewater (Samsudeen and Matheswaran, 2018), and treatment and energy recovery from the main process stream of domestic wastewater treatment plants(Escapa et al., 2012; Gil-Carrera et al., 2013; Heidrich et al., 2014, 2013). Large scale (<100L) MECs have been tested with domestic wastewater and been shown to remove up to 65.6% of chemical oxygen demand from raw wastewater at a hydraulic retention time of one day (Heidrich et al., 2014). To the author's knowledge, there has been no work evaluating the application of MECs to high strength solids-dewatering wastewater for wastewater treatment and energy recovery. This work tests a new liter-scale platform and investigates the relative performance of MECs fed acetate media, primary effluent, solids-dewatering filtrate, and a 60:40 (primary effluent: filtrate) feedstocks by biogas energy yield and treatment energy savings.

Materials and Methods

Reactor Construction and Configuration

MECs consisted of plastic aquariums (23.19(L) x 16.82-cm(W), 3.5 liters volume) sealed between two acrylic plates (30.48-cm (L) x 20.32-cm (W). A rubber gasket (24.13-cm (L) x 15.875-cm (W)) was cut and placed between the top acrylic plate and the top rim of the reactor body to act as a gas seal. Stainless steel bolts, wing nuts (.9525-cm), and flat washers (.9525-cm) were placed through holes of the top and bottom acrylic blocks to form a pressure seal. The anodes were carbon fiber brushes with titanium center wire (2.54-cm diameter, 12.7-cm (L), 11.43-cm brush section) fitted in the drilling holes (.2778-cm diameter) on the top acrylic block. The cathode was a rectangular sheet of stainless steel SS 304 90 x 90 mesh (McMaster-Carr, New Jersey USA) (17.78-cm (L) x 12.065-cm (W)) with stainless steel wire leads through the top cover plate. The effective surface area of the cathode was calculated as 0.432m^2 based on a calculated real to geometric surface area ratio of 20.15:1 (Zhang et al. 2010). A cylindrical glass tube (1.27-cm diameter) was epoxied on the top of the acrylic block and crimped with a butyl rubber stopper and aluminum cap (20-mm). A needle placed through the butyl rubber stopper connected through a length of tubing (60.96-cm), to a gas collection bag. Over the course of the experiment, MECs were intermittently submerged in water and pressurized using hand pressure on a "dummy" gas bag to detect leaks.

Start up and Operation

The experiment consisted of three pairs of MECs and open circuit controls operating with different wastewater feedstocks as well as a sodium acetate positive control MEC for a total of four closed circuit MECs and seven systems. Reactors were operated in fed-batch mode, with weekly fill cycles. Wastewaters tested in this study included primary effluent, a high-strength final solids dewatering liquor ("filtrate"), and a 60:40 (primary:filtrate) ratio. Wastewater samples were collected fresh the day of

refilling and transported from a large advanced wastewater treatment plant in the US Mid-Atlantic region. The positive control consisted of a commonly used 1000ppm sodium acetate in 50mM PBS solution (sodium acetate (1 g/L), Na_2HPO_4 (4.08 g/L), NaH_2PO_4H2O (2.45 g/L), NH_4Cl (.31 g/L), and KCl (.13 g/L)). Weekly refilling of reactors through the gas sampling port consisted of the removal of the crimp seal and butyl rubber stopper, draining of the reactors via a siphon, and immediate refill of the reactors with fresh media and replacing of the stopper and crimp seal. By this method, the authors estimate that the interior of the reactor and electrode biofilms were exposed to the atmosphere for less than 5 minutes once a week.

The MEC modules were electrically connected with an established method (Call and Logan, 2011), where all shared a single power supply (model XP-752A; Elenco Electronics Inc., Ohio USA) which fixed a cell potential of 1.0V, with a 0.1 Ω resistor in line with the positive lead. Voltages across the external resistance were measured at 5 minute intervals with a digital multimeter (model 2700; Keithley Instruments Inc). Current was calculated according to Ohm's law and normalized to cathode effective surface area. Early tests used a 10Ω resistor in line with the positive lead, but large voltage drops across this resistor (>0.1V) greatly affected the applied voltage experienced by the MECs. In accordance with Ohm's law, MECs which produced more current experienced less applied voltage. While this may have not been significant to influence experiments with the small current passing through the circuit in the Call and Logan method, a liter-scale system required a smaller external resistance to minimize differences in applied voltages in this study. For this reason, the voltage, current, and power data reported herein is based on voltage measurements across a 0.1 Ω resistor.

Water Treatment Analysis

Total and soluble chemical oxygen demand (COD) were performed at the start and end of each cycle . The soluble COD were filtered with a 0.45 μm filter. For the influent total and soluble COD the primary filtrate and the PBS used a standard method (21259 vial Digestion Solution for COD HR 20-1500 mg/l Range; Hach Company) with 2 mL of samples.

Biofilm Analysis

DNA will be extracted from mature biofilm samples on anode, cathode, and reactor vessel surface using a commercially available kit (PowerSoil DNA Isolation Kit, MoBio) and Illumina sequencing of the V3-V4 region of the 16s rRNA gene will be performed. Data will be processed through the DADA2 pipeline to determine relative abundance of taxa and perform alpha and beta diversity analyses.

Energy Balance

The energy benefit of MEC implementation can be described as the sum of energy contained in produced biogas and treatment energy savings less the energy input. The energetic recovery is determined by quantifying the volumetric biogas yield and composition and multiplying by the heat of combustion. The treatment energy savings is described as the opportunity cost of COD removal, described as 3.2 $kg * kg^{-1}$ (Wan et al., 2016).

Hydrogen and methane mole fractions of gas in headspace and gas bags were determined using a GC-TCD method. At the time of writing, the authors are working to improve to improve the accuracy of hydrogen quantification. The method for quantification of hydrogen and methane yield is based on an established method (Ambler and Logan, 2011). In this method, the mole percentage of hydrogen and methane accumulated in a gas bag over a reactor fill cycle is determined before and after the addition of a known volume of nitrogen. A mass-balance analysis can then be performed to determine volumetric yields of hydrogen and methane.

Results / Discussion

MECs fed acetate solution produced the highest current density, followed by the 60:40 blend, filtrate, and primary effluent. This may be explained by increased conductivity and chemical oxygen demand in filtrate

The difference in sCOD removal between open and closed circuit replicates was more pronounced in higher strength feedstocks. The filtrate MEC removed 283 ± 117 mg/L of sCOD as compared to 13 ± 86 mg/L sCOD in the open-circuit replicate. However, the primary effluent MEC removed a comparable quantity of sCOD as compared to its open circuit replicate (Table 1), meaning that the native consortia to the media was effective at oxidizing the organic material. In the filtrate MEC, where the feedstock is mostly recalcitrant organics that survived a 28-day anaerobic digestion process, the native consortia was not able to remove as much of the sCOD (1 ± 6 %). However, in the closed-circuit MEC containing electrochemically active biofilms, 18 ± 7% sCOD was removed. This suggests that MECs are capable of enhancing the biodegradation of recalcitrant organics and may be effective at removing organics that other processes do not.

This system did not recover detectable quantities of hydrogen in the produced biogas when fed wastewater, but did when fed acetate media. Gas accumulated in the collection bag of all closed-circuit MECs and contained methane. It is possible that hydrogen was not produced at the cathode of these reactors and a different chemical served as the terminal electron acceptor, or this could be the result of consumption of produced hydrogen by microbes growing on the cathode or in the media. There is development of a visible biofilm layer on the cathodes, and it is hoped that biofilm sampling and community analysis will help to elucidate the transformations occurring at the cathode. It has been observed in other single-chamber MECs that hydrogen produced at the cathode can be re-oxidized at the anode, creating current not associated with the degradation of organics, deceptively large coulombic

efficiencies and reducing hydrogen yield from the system (Lee et al., 2009). In this system where the acetate fed MEC is producing hydrogen in collected biogas but the wastewater fed MECs are not, it suggests the possibility that hydrogen is being consumed by planktonic microbes in the media. Filtrate contains a microbial consortia from an anaerobic digestor system where hydrogen is used as an electron donor in methanogenesis, so there is likely a robust hydrogenotrophic community present in the media. This suggests that a two-chamber membrane system, or a separator with a separate catholyte (Heidrich et al., 2014) may be required for effective hydrogen recovery from domestic wastewater. It has also been demonstrated that methanogens are much more sensitive to oxygen exposure than anode-respiring bacteria, and that periodic exposure to oxygen can greatly impair methanogens while only slightly suppressing anode-respiring bacteria (Chae et al., 2010). Due to the method of emptying and refilling the large reactors in this study through the gas sampling tube in quick succession, it is unlikely that biofilms inside the reactor were ever exposed to atmospheric oxygen.

Conclusion

The MECs presented in this study were effective at removal of sCOD but were not effective in recovering energy as hydrogen in biogas. This suggests that a separator or method of methanogen inhibition may be effective in increasing the purity and yield of hydrogen from wastewater MECs. MECs were shown to increase the rate of sCOD removal in high-strength wastewater.

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Conclusions and Future Recommendations

Both bioelectrochemical methods evaluated in this work show promise for removal of organic contaminants from domestic wastewater process streams. However, the much higher electron recovery efficiency of MECs than MFCs suggests that they may have a more effective role in ability to recover energy or resources from wastewater.

Future research should focus on improving system architecture with the goal of optimizing net-energy benefit. Analysis of temporal and spatial biofilm composition patterns coupled with power production and treatment data will help to understand which portions of the large cathode electrodes used in the pilot study are most efficacious at recovering energy and providing treatment energy savings. This information will be used to design and configure electrodes in future systems in order to operate upscaled MFCs with effective biocathodes. Design and function of MFC biocathodes is still poorly understood and before this work had not been characterized on an upscaled system. A recent study comparing the power densities of acetate MFCs with biocathodes and conventional platinum catalysts suggested that a "biohybrid" cathode with both applied chemical catalyst and biofilm growth outperformed both a biocathode and an abiotic platinum-doped cathode (Massaglia et al 2019). If a similar approach is sucessful with a wastewater MFC, increases in power density could be achieved through the addition of only a small quantity of chemical catalyst. Alternately, pre-acclimated biocathodes could be cultivated through initial polarization using a potentiostat.

Nitrogen removal from wastewater was not addressed in the current reported work but could be of great value to the wastewater treatment industry which currently uses a lengthy and expensive process and is strained by increasingly stringent discharge permits. Analysis of spatial biofilm distribution in the pilot cathode brushes may reveal certain regions which host higher relative abundance of nitrogen-transforming bacteria. In this case, an improved system could be engineered to take advantage of these niches. Another possibility is to use potentiostatically controlled electrodes to achieve nitrification with an

electrode as an electron acceptor and denitrification with the electrode serving as an electron donor. Membrane based systems are able to recover ammonia via volatilization in a high pH cathode chamber, which may be significant from dewatering process streams with ammonia nitrogen concentrations greater than 1500 mg/L.

The MEC system tested in this work showed promise for the removal of chemical oxygen demand from domestic wastewater. In particular, it outperformed the anaerobic treatment control with higher strength wastewater blends. This design could be improved by increasing the electrode packing density within the reactor. Gas recovery was poor in comparison with the theoretical production based on charge passed through the circuit, and hydrogen was not recovered from wastewater, only from the acetate positive control. Higher methane was recovered in the headspace from closed circuit wastewater than anaerobic controls, suggesting that the MEC treatment has the potential to increase the rate of methanogenesis, or promote additional methane production from "exhausted" digestor effluent.

In addition to providing insight into the applicability of bioelectrochemical systems to real wastewater process streams, this work resulted in the creation of and investment in a pilot test location at DC Water. Continued operation of the MFC pilot at DC Water will provide important data on the long term treatment and energy recovery potential of scaled-up MFC systems and also provide insight into the temporal dynamics of biofilm community structures. The current system could also be modified to test the applicability of several other bioelectrochemical systems. For example, the existing modules could be converted to an MEC rather simply by connecting the pre-acclimated electrodes across a power supply and placing a cap on the tank to collect biogas. Other electrochemical treatment and nutrient recovery systems could also be tested in this space including electroactive anammox or volatilized ammonia recovery. The current system represents an initial step towards developing scaled-up bioelectrochemical resource recovery systems at DC Water and sets the framework for varied and promising future work.

Appendix A: Bioinformatic analysis of biofilm samples from Muddwatt reactors operated with different wastewater feedstocks

Reactors operated and 16s rRNA sequencing results from unpublished work by Ko, Wing-Mei and Kjellerup, Birthe, 2016.

Read processesing and figures produced by Leininger, Aaron, 2018 using DADA2 pipeline in RStudio.

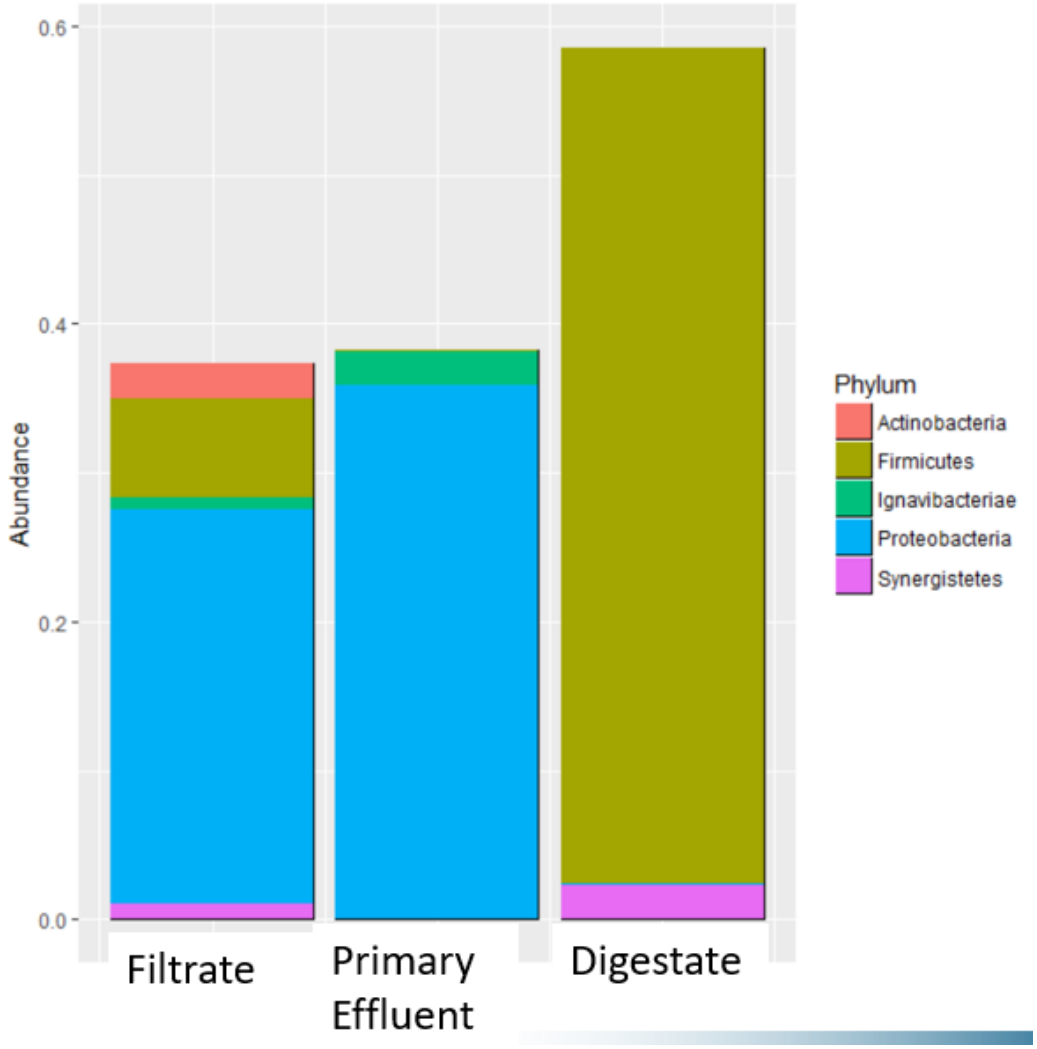
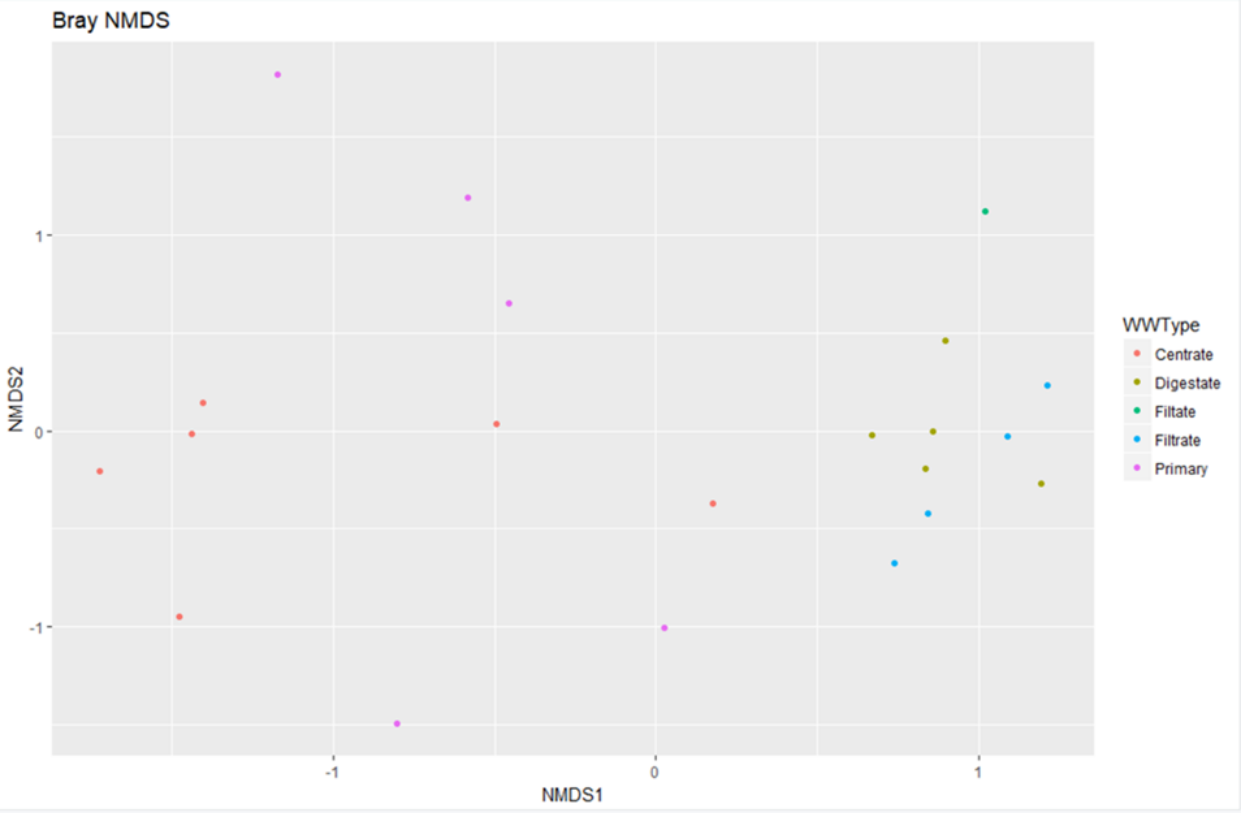


Figure s1: Alpha diversity of anodic biofilm communities in microbial fuel cells operated with primary effluent, filtrate, and digestate in a preliminary study



Figures2: Nonmetric Dimensional Scaling of Biofilm communities associated with initial, final, and anodic biofilms of microbial fuel cells operated with centrate, digestate, filtrate, and primary effluent.

Appendix B – Supplementary Information to Pilot MFC System

Figure s1: Pilot reactor system at DC Water Blue Plains, Nov 29 2018

Figure s2: Section of cathode module during flow-through operation, DC Water Blue Plains Nov 29 2018

Figure s3: Source of filtrate for pilot MFC system, filtrate reactor feed head tank at Filtrate Treatment Facility, DC Water Blue Plains, Nov 29 2018

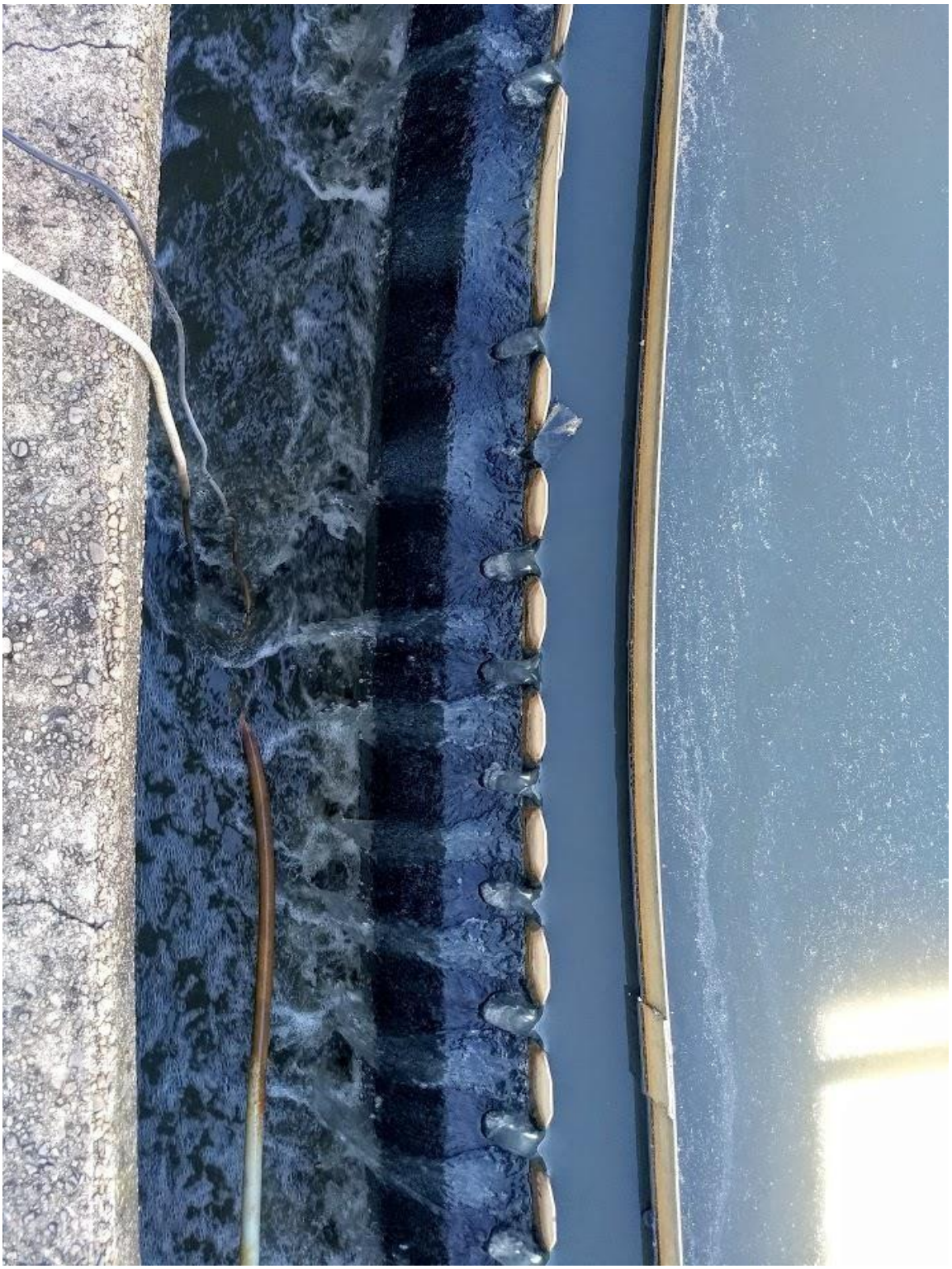


Figure s4: Source primary efluent for pilot MFC system, submersible pump in primary effluent channel of a west primary clarifier, DC Water Blue Plains, Nov 29 2018

Figure s5: Intermediate holding/constant head overflow tank (1000L IBC Tote) for primary effluent, DC Water Blue Plains, Nov 29 2018. Primary effluent enters from top, pumped to pilot from top, overflows from dual lines. Sediment periodically emptied from bottom drain.

Figure s6: Duplicate of fiberglass tank used for MFC reactor. DC Water Blue Plains, Nov 29 2018.

Figures7: An MFC module removed from tank for biofilm sampling at end of open-circuit operation. DC Water Blue Plains, Oct 8 2018.

Figures 8: An electrode brush being prepared to seal with electrical insulating epoxy. DC Water Blue Plains, Aug 31 2018.

Figure s9: Converted shipping container used to house MFC pilot reactor. DC Water Blue Plains, Nov 29 2018.

Figure s10: Arrangement of electrodes during cyclic voltammetry tests . DC Water Blue Plains, Sept 12 2018.

Figure s11: Brush lattice created and used as counter electrode for cyclic voltammetry tests . DC Water Blue Plains, Sept 7 2018.

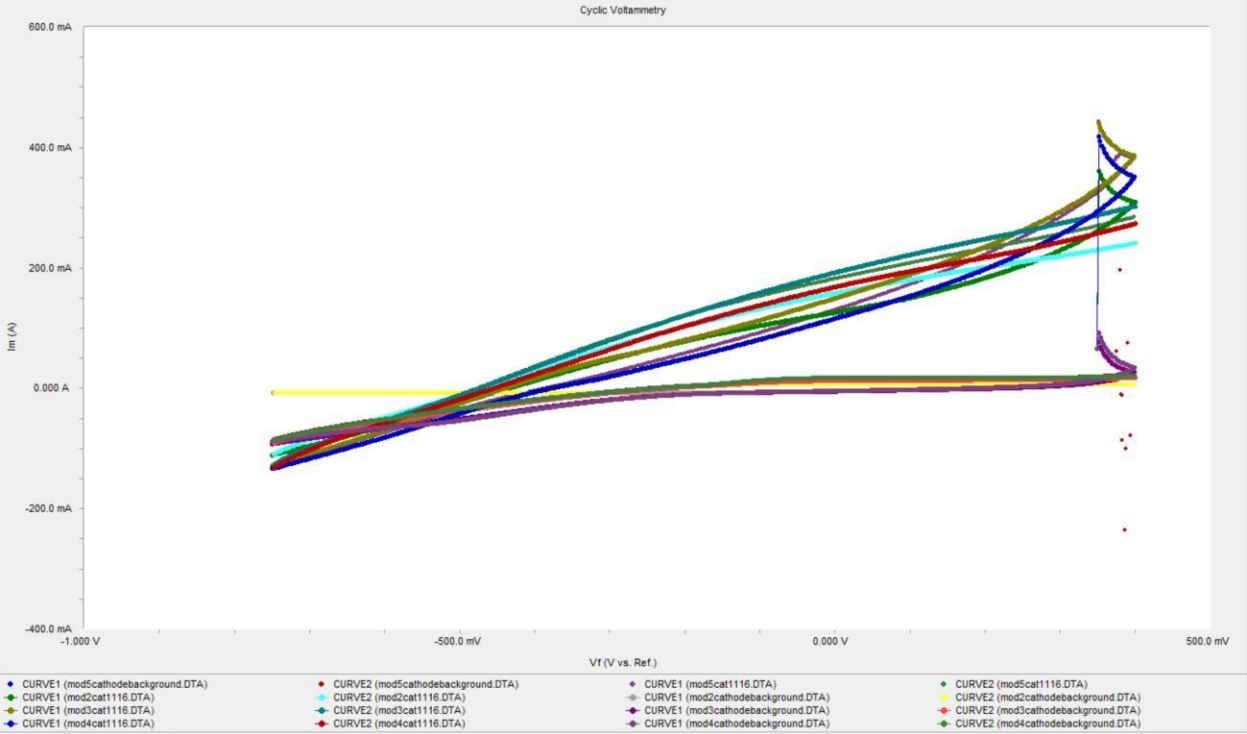


Figure s12: Cyclic Voltammagrams of background and acclimated cathodes from all modules

Appendix C – Supplementary Information to Bench MEC System

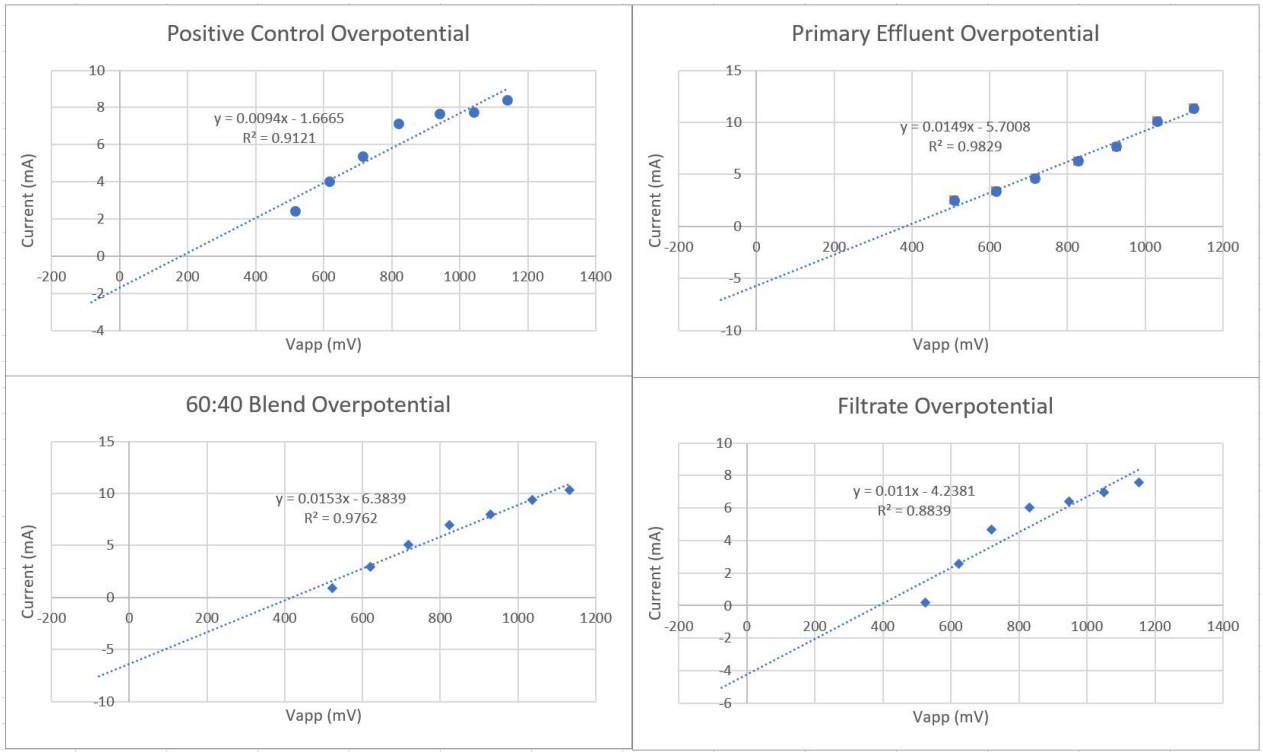


Figure s1: Overpotential plots from four closed-circuit MECs operating with different feedstocks

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