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SCREENING AND ACCLIMATION METHODS FOR ACCOMPLISHING TREATMENT AND ENERGY RECOVERY FROM WASTEWATER IN MICROBIAL ELECTROLYSIS CELLS

A Thesis in

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by

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ABSTRACT

Microbial electrolysis cells (MECs) are an emerging bioelectrochemical technology that can recover energy from organic matter in wastewater. In an MEC, a biological anode populated with microbes, capable of oxidizing organic compounds and generating an electrical current, is paired with a conductive, hydrogen-evolving cathode. Wastewater composition and concentration can vary significantly between sources, which influences organic treatment, gas production, and current generation in MECs. Inexpensive, miniature MECs (5 mL) have been previously used to examine MEC performance with different industrial effluents, but they have not been compared with more widely used reactor designs. In this study, mini MECs and larger cube MECs (32 mL) were compared with industrial (IW), domestic (DW), fermentation (FE), and synthetic (AC) effluents to better understand how performance corresponds between these reactor designs.

Before MEC treatment, the IW, DW, FE and AC samples contained 450-4500 mg/L of chemical oxygen demand (COD) and 230-800 mg/L of biochemical oxygen demand (BOD). 66-92% of COD was removed in MECs for all samples, with higher average current density observed with the AC and FE samples (2.25-6.72 A/m^2), which were buffered, than the IW and DW samples (0.64-1.93 A/m^2). COD removal and coulombic efficiency (CE) correlated well between mini and cube MECs. Total charge (normalized to the reactor liquid volume) and the rate of current generation were similar between mini and cube MECs fed well-buffered samples (AC and FE), but significantly different for industrial and domestic effluents (IW and DW). Mini MECs were found to suitably represent cube MEC treatment performance and are useful for screening real wastewaters for potential larger scale MEC treatment.

Different acclimation procedures were also investigated with cube and mini MECs to determine the influence on current generation, organic removal, and gas recovery with

fermentation effluent as substrate. Pre-acclimating MEC biofilms with domestic wastewater or acetate medium prior to treating fermentation effluent slightly improved COD removal (3-5%), compared to non-pre-acclimated reactors, but gas production and current generation were unchanged by the acclimation method. Differences in protein removal were relatively small between acclimation methods in mini MECS (<5%), with no difference measured in cube MECs. Although pre-acclimation improved treatment in both mini and cube MECs, the difference in COD treatment, current generation, and protein removal was more significant between mini and cube MECs than acclimation methods. These results suggest that acclimation method has a relatively small influence on MEC performance and acetate addition may not be necessary to develop a robust electrically active biofilm.

TABLE OF CONTENTS

LIST OF FIGURES

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1. INTRODUCTION

Emissions from the combustion of fossil fuels have altered the Earth's climate and dramatic environmental changes are expected in the next century if current energy practices are maintained. Renewable and efficient energy infrastructure is needed to mitigate the negative impacts of climate change. Currently, the majority of electricity produced and consumed in the U.S. is generated using fossil fuels. Abundant natural gas and coal resources are used to produce more than half of the electricity generated in Pennsylvania (EIA, 2014). Wastewater treatment plants consume about 15 GW, or approximately 3 percent, of the total electricity demand in the U.S., and capacity will need to increase with population in the coming decades (Logan & Rabaey, 2012; Water, 2006). Aerobic technologies are widely used to treat industrial, agricultural, and domestic wastewater and are very effective at removing nutrients that can be harmful if released into the environment. These aerobic treatment systems typically do not utilize the significant energy (5.6-16.8 kJ/L for domestic wastewater) contained in the organic material present in many effluent sources (Heidrich et al., 2011). Wastewater is an untapped energy and nutrient source that could be used to offset treatment energy, decrease electricity demand, and reduce greenhouse gas emissions from fossil fuels.

Hydrogen gas is used in many industrial applications, including fertilizer and chemical production, as well as petro-chemical refining (EIA, 2008). Over 95 percent of hydrogen production comes from fossil fuel sources, with nearly half from steam reforming of natural gas (Logan, 2004; Olah et al., 2009). This accounts for approximately 2 percent of primary energy usage in the U.S. (Keith & Farrell, 2003). The proliferation of cheap natural gas and coal in the U.S. facilitates these methods of hydrogen production, but fossil fuel resources are finite and alternative methods will eventually become necessary to meet the demand for hydrogen.

Microbial electrolysis cells (MECs) could potentially be used to reduce fossil fuel emissions associated with electricity demand for wastewater treatment and hydrogen production. MECs are similar to microbial fuel cells (MFCs), in that they both utilize microorganisms that oxidize organic material anaerobically and transfer electrons to a conductive anode (Call & Logan, 2008; Liu et al., 2005; Rozendal et al., 2006). In an MEC, electrons travel through an external circuit and recombine with protons at the cathode to evolve hydrogen, instead of combining with oxygen to form water like in an MFC. The whole cell reaction in an MEC is not thermodynamically favorable and does not proceed spontaneously, so an additional 0.11 V is theoretically required to drive the system with acetate as a substrate (Liu et al., 2005; Rozendal et al., 2006). In practice, an applied potential of 0.5 V or greater is typically needed to drive hydrogen evolution at a reasonable rate due to electrode over potentials and internal resistance (Logan et al., 2008).

MEC performance is dependent on a variety of factors within the reactor, including pH, conductivity, substrate composition and biological degradability (Logan et al., 2008; Rozendal et al., 2008). Volatile fatty acids (VFAs), like acetate, are easily degraded by exoelectrogens, but real wastewaters are more complex and may contain a mix of carbohydrates, fats, proteins, and other less degradable material (Rozendal et al., 2008). Complex effluents have been tested in a wide range of reactor designs, including pilot scale MECs (Cusick et al., 2011; Heidrich et al., 2013), but the cost to build and operate larger reactors makes it difficult to evaluate a large number of different effluent samples. Direct performance measurements in MECs are necessary because typical wastewater metrics used to quantify organic concentration and aerobic biological degradability, like chemical and biochemcial oxygen demand, do not accurately reflect treatment or energy recovery potential in MECs (Ren et al., 2013). A recently developed high-throughput,

inexpensive, miniature MEC design has been used to evaluate treatment performance with complex wastewater samples (Call & Logan, 2011; Ivanov et al., 2013; Ren et al., 2013), but performance in mini MECs has not been directly compared to larger bench scale reactor designs that allow for more comprehensive analysis.

The objectives of this study were to determine if the performance of high-throughput mini MECs is comparable to that obtained in larger reactors (with different materials), and if different methods of anodic biofilm enrichment influence MEC performance and protein removal with a complex lignocellulose fermentation effluent. To meet the first objective, treatment performance and current generation in mini MECs was compared to larger cube MECs when both were operated with real or synthetic wastewater samples. This comparison provided insight into the utility of high-throughput mini MECs for evaluating potential energy recovery and treatment of different wastewater sources. The second objective was met by pre-enriching anodic biofilms in mini MEC reactors using domestic wastewater (DW) or buffered acetate medium and comparing performance when switched to fermentation effluent. Anode enrichment in cube MECs was investigated by starting reactors in MFCs fed DW and transferring to MECs. Both mini and cube MEC acclimation results were compared with reactors that were fed fermentation effluent without anode pre-acclimation. The results from these tests can be used to provide guidance on optimal start-up methods for full scale MECs treating real wastewaters.

2. LITERATURE REVIEW

Successful implementation of MECs for energy recovery and treatment of waste streams is dependent on building reactors capable of utilizing organic matter from domestic, industrial, and agricultural effluents at a cost that is comparable to current treatment methods. Although MECs have only been researched for a decade, material and reactor design improvements have rapidly increased performance and shown the technology can be sustained and efficient at both small and larger scales (Logan, 2010). While there continues to be progress in improving reactor design and efficiency, finding and evaluating suitable effluent sources is equally important for further development of cost effective treatment systems (Logan & Rabaey, 2012).

2.1 Exoelectrogenic Microbes

A significant number of microbes have shown the ability to produce power in bioelectrochemical systems, both as pure cultures and diverse mixed cultures (Logan, 2009). Known as exoelectrogens, these current generating microbes are prevalent in the environment and anodic biofilms in bioelectrochemical systems (BESs) have been inoculated with aerobic and anaerobic wastewater, river water, soil, and estuarine sediments (Jiang et al., 2010; Ki et al., 2008; Yates et al., 2012). *Geobacter sulfurreducens* is known to produce current in pure culture tests, and this or similar strains are often prevalent in mixed culture anodic biofilms with good current production (Logan, 2009). In MECs inoculated with domestic wastewater and fed acetate, *G. sulfurreducens* dominated the anodic community, even though it was not as abundant in the inoculum (Call et al., 2009). *G. sulfurreducens* was also a primary community component in MECs inoculated and fed domestic or winery wastewaters, although the overall communities were more diverse than those found in acetate fed reactors (Cusick et al., 2010). Organic

substrate and inoculum influence the microbial community in an MEC, which effects current generation, treatment performance and gas conversion efficiency.

Developing anodic communities in MECs prior to treating complex or biologically inactive substrates improves treatment performance and reduces cycle time (Ivanov et al., 2013; Ren et al., 2013). Domestic wastewater provides a complex microbial inoculum and an organic substrate, and it has been used to develop anodic biofilms in MECs before the reactors are switched to other substrates. Acclimating MECs to domestic wastewater prior to treating an industrial wastewater sample reduced batch cycle time to 4 days, compared to 10 days for reactors that were not pre-acclimated, with similar chemical oxygen demand (COD) removal and average current (Ivanov et al., 2013). Refinery wastewater treatment with MECs also benefitted from acclimation to domestic wastewater, with reduced start-up time and enhanced organic removal due to the pre-developed anodic biofilm (Ren et al., 2013). Acclimating reactors to single components of a synthetic fermentation effluent and then using that effluent to inoculate MECs treating the synthetic effluent improved hydrogen production and efficiency, compared to reactors acclimated to just the synthetic effluent, indicating that inoculum source had a significant impact on treatment performance (Lalaurette et al., 2009).

2.2 MEC Materials

The anode in an MEC serves as a platform for the electrically active biofilm. To facilitate electron transfer from microbes to the anode, the material must be conductive, have a large specific surface area, and enable strong microbial adhesion (Logan et al., 2008; Wrana et al., 2010). Carbon materials, including carbon cloth, carbon paper, graphite felt, graphite granules, graphite blocks, and graphite fiber brushes, meet most of these requirements and all have been used as anodes in BES studies (Call & Logan, 2008; Ditzig et al., 2007; Liu et al., 2005; Logan

et al., 2007; Tartakovsky et al., 2009). Carbon fiber brushes have shown high current densities and hydrogen production rates in MECs, they have a very high specific surface area that readily sustains a large biofilm mass, and they cost less than other electrode materials, like fuel cell grade carbon cloth (Call & Logan, 2008; Logan et al., 2007; Logan, 2010). Carbon brushes have also been successfully used in a pilot MEC, showing their potential for use in larger scale systems (Cusick et al., 2011).

The hydrogen evolution reaction (HER) occurs at the cathode in an MEC as electrons generated at the anode combine with protons to form hydrogen gas. A catalyst is typically used on the cathode surface because of high over potentials and low reaction rates on plain carbon electrodes (Logan, 2007; Logan et al., 2008). Platinum is known to be a good HER catalyst and has been used extensively in MEC experiments, although it is expensive and therefore not practical for larger scale MEC applications (Logan, 2007; Selembo et al., 2010). Nickel, stainless steel, and molybdenum disulfide are promising low-cost alternative cathode catalysts that have been previously tested in MECs (Selembo et al., 2010; Selembo et al., 2009a; Tenca et al., 2013). Powdered nickel has an overpotential that is lower than stainless steel, and close to that of platinum, and it has produced current densities and hydrogen at rates that are close to platinum catalysts under optimal conditions (Selembo et al., 2010). Platinum outperformed both molybdenum disulfide and stainless steel cathodes in MECs with food processing and industrial wastewater as substrate, although gas production and COD removal were improved with a molybdenum disulfide catalyst layer, compared to stainless steel mesh (Tenca et al., 2013). The differences in gas recovery, organic removal, and current generation between MECs fed food processing or industrial wastewaters were more significant than the differences between cathode catalysts (Tenca et al., 2013). Catalysts are important for promoting efficiency, but substrate

concentration and composition may be more important factors when operating MECs with real wastewaters.

Separating the electrode chambers in an MEC with an ion selective membrane increases H_2 gas purity, reduces methanogenesis, and reduces cathode fouling, but contributes to a high internal resistance that reduces current densities and hydrogen evolution rates (Logan et al., 2008). Using a cation exchange membrane (CEM) limits hydrogen gas diffusion into the anode chamber while allowing proton transport to the cathode, but its use reduces performance by creating a pH imbalance between the anode and cathode chambers, since all cationic species, not just protons, can be transferred to the cathode (Logan et al., 2008). Using an anion exchange membrane (AEM) improves the pH imbalance, but removing the membrane altogether eliminates the internal resistance generated by the membrane and improves hydrogen production rates (Call & Logan, 2008; Cheng & Logan, 2007; Hu et al., 2008).

2.3 Organic Substrates

MECs can be used to recover energy from a wide variety of simple and complex organic substrates (Logan, 2009; Logan et al., 2008). Simple organic compounds, like acetate, glucose, and certain volatile fatty acids, are efficiently degraded by exoelectrogens, producing current and enabling hydrogen gas production (Lalaurette et al., 2009; Logan et al., 2008; Selembo et al., 2009b; Tartakovsky et al., 2009). In MEC experiments, these substrates are typically used in buffered solutions to limit pH changes and increase conductivity, which reduces electrode overpotentials and ohmic losses (Rozendal et al., 2007; Rozendal et al., 2008). Phosphate buffered acetate media have been widely used in MEC studies comparing different reactor designs and materials. Although acetate media is useful for MEC studies since the composition remains consistent, it does not represent the complex and variable nature of real wastewaters that

can be poorly buffered or have much lower conductivities (Cusick et al., 2010; Rozendal et al., 2008).

The organic concentration, composition, buffering capacity and conductivity of real wastewater can vary significantly between sources, which can influence treatment and energy recovery in MECs (Cusick et al., 2010; Tenca et al., 2013). A wide range of real wastewater samples have been evaluated in MECs, including refinery, swine farm, food processing, domestic, landfill leachate, winery, fermentation, and various industrial effluents (Cusick et al., 2010; Ditzig et al., 2007; Escapa et al., 2012; Ivanov et al., 2013; Mahmoud et al., 2014; Ren et al., 2013; Tenca et al., 2013; Wagner et al., 2009). Initial COD concentrations in these complex samples can range from around 300-400 mg/L for domestic wastewater, up to 12,000-17,000 mg/L for swine farm effluent (Cusick et al., 2010; Wagner et al., 2009). Current and hydrogen generation vary significantly between wastewater samples, even those from different locations within the same facility (Ren et al., 2013). Biogas production rates of 1-2 m^3/m^3-d have been observed using complex substrates, like industrial and swine farm wastewaters, although methane can comprise a large fraction of the recovered gas in single chamber systems (Tenca et al., 2013; Wagner et al., 2009). Effluent COD also varies, but is typically high enough that additional treatment would be required before discharge, so MECs may not be suitable as a single stage treatment system.

Solid lignocellulose waste materials, like those generated by timber milling, paper production, and agriculture, can also be converted into hydrogen through biological fermentation (Lee et al., 2010; Levin et al., 2004). Dark fermentation is a biohydrogen production process that can utilize carbohydrates from cellulosic material, but the hydrogen yield can be limited by the buildup of volatile fatty acids and the effluent is highly enriched in soluble organic material (Lee

et al., 2010; Levin et al., 2006; Magnusson et al., 2008). Of the 12 mol H_2/mol glucose that can be theoretically generated using dark fermentation, only 2-3 mol H_2/mol glucose are extracted in practice (Liu et al., 2005; Logan, 2004; Valdez-Vazquez & Poggi-Varaldo, 2009). MECs have been proposed as a method to further treat dark fermentation effluent and extract more hydrogen since exoelectrogens can readily degrade VFAs and other organic material present in fermentation effluent (Hawkes et al., 2007; Logan et al., 2008). A two-stage process combining dark fermentation and MECs yielded 9.95 mol H_2/mol glucose equivalent using cellobiose as a feed source, which was a significant improvement over the 1.64 mol H_2/mol glucose equivalent obtained with dark fermentation alone (Lalaurette et al., 2009).

2.4 Hydrogen Cycling and Methanogenesis

Methane production is a common problem in mixed culture MECs, especially for reactors operated in continuous flow mode, since methanogenic microbes are prevalent under the same conditions as exoelectrogens (Lee et al., 2009; Rader & Logan, 2010; Tartakovsky et al., 2009). Acetoclastic methanogens directly convert acetate into methane, but are generally out competed by anode respiring microbes in MECs (Lee et al., 2009; Parameswaran et al., 2009). Hydrogenotrophic methanogens can convert hydrogen generated at the cathode and produced from substrate fermentation into methane, with bicarbonate as a carbon source (Cheng et al., 2009; Lee & Rittmann, 2010; Parameswaran et al., 2009). Hydrogen is relatively insoluble under normal MEC operating conditions (K_H=7.66×10^{-4} mol/L-atm at 30°C, 1 atm), so methane production in acetate fed MECs typically increases as hydrogen accumulates in the solution and headspace, leading to higher methane concentrations as hydraulic retention time increases (Call et al., 2009; Chemical Rubber; Lee & Rittmann, 2010).

Methanogenic activity is difficult to suppress in MECs since methanogens can proliferate throughout the reactor and are not limited to colonization of the anode. In anaerobic digestion, low pH caused by VFA buildup can inhibit methanogens, but exoelectrogens in MECs are also inhibited by low pH (Clauwaert & Verstraete, 2009; Wagner et al., 2009). Exposing the anode to air between cycles in fed batch reactors can limit methane generation, but does not completely eliminate it (Call & Logan, 2008). Very low hydraulic retention times in continuous flow MECs also do not fully eliminate methanogens, indicating that they are primarily attached in the cell and not in suspension (Clauwaert & Verstraete, 2009; Lee & Rittmann, 2010). Methanogenesis can be chemically inhibited, but this approach is not sustainable or practical for large-scale systems (Lee et al., 2009). Although methane is not as valuable as hydrogen, it is still a potent energy carrier. Instead of suppressing methane production, electromethanogenesis could be another method of extracting energy from wastewater (Cheng et al., 2009; Clauwaert et al., 2008).

Hydrogen can also be used as an electron donor by anodic microbes in single chamber MECs, which results in hydrogen cycling between the electrodes. This generates artificially high current and decreases energy efficiency, since the applied energy increases with current (Lee et al., 2009). In a continuous flow single chamber MEC, hydrogen cycling accounted for over 60% of the observed current, increasing the CE to over 190%, while the cathodic conversion efficiency remained below 25% (Lee & Rittmann, 2010). Separating the anode and cathode with a selective membrane helps to eliminate hydrogen cycling, but increases internal resistance and system cost.

2.5 MEC Operation and Scaling

MEC pilot scale reactors operated with complex substrates have shown potential for wastewater treatment, but these studies have also shown some of the limitations and shortcomings of larger-scale implementation relative to laboratory-based results. A 1000 L continuous flow MEC treating winery wastewater removed approximately 60% of soluble COD and produced 0.15-0.30 L/L-day of biogas, but methane (rather than hydrogen gas) was the dominant gas product (Cusick et al., 2011). Another pilot-scale MEC (100-L, continuous flow) was used to treat domestic wastewater, and it generated hydrogen gas with very little methane, but the rate was less than 0.025 L/L-day with only 70% of the input electrical energy recovered in the biogas (Heidrich et al., 2013). COD removal was also variable and did not consistently meet discharge standards, indicating that post-treatment after a full scale MEC system may be necessary.

Chemical and biochemical oxygen demand are typical measurements used to quantify the organic concentration and biodegradability of wastewaters, but they do not accurately represent MEC performance potential. Direct measurements in MECs are necessary to evaluate performance with various wastewater sources (Ivanov et al., 2013; Ren et al., 2013). Bench scale reactors have been used to study the performance of various substrates and can provide valuable treatability information for larger scale MEC implementation. A 0.03 L reactor fed winery wastewater produced current densities that were reasonably similar to a 1000 L pilot scale reactor, even though there were material and operational differences between the reactors (Cusick et al., 2011; Cusick et al., 2010). Typical bench scale reactor designs are very useful, but reactor parts and equipment, such as multiple power supplies, machined reactor parts, and platinum catalysts, add to the cost of operating multiple reactors and limit their application for substrate screening.

Mini MECs are a high-throughput reactor design that are small, cost less than $2 each, and can be operated with a large number of reactors in parallel on a single power supply (Call & Logan, 2011). They have been used previously to screen industrial effluent sources to determine potential BES applications, but performance has not yet been directly compared with larger MECs (Ivanov et al., 2013; Ren et al., 2013).

3. TREATABILITY OF COMPLEX EFFLUENTS IN HIGH-THROUGHPUT AND BENCH SCALE MICROBIAL ELECTROLYSIS CELLS

3.1 Abstract

High-throughput mini microbial electrolysis cells (MECs) were compared with larger, bench-scale cube MECs using a range of wastewaters and substrates. COD removals and coulombic efficiencies corresponded well between the two reactor designs for individual samples, with 66-92% of COD removed for all samples. Current generation was consistent between the reactor types for acetate (AC) and fermentation effluent (FE) samples, but less consistent with industrial (IW) and domestic wastewaters (DW). Hydrogen was recovered from all samples in cube MECs, but gas composition and volume varied significantly between samples. Evidence for direct conversion of substrate to methane was observed for two of the industrial wastewater samples (IW-1 and IW-3). Mini MECs provided organic treatment data that corresponded well with larger scale reactor results, and therefore they can be a useful platform for screening potential wastewater sources.

3.2 Introduction

The organic matter present in wastewater is an energy and nutrient rich resource that is currently under-utilized. Aerobic wastewater treatment methods consume a significant amount of energy (~0.6 kWh/m^3) and have limited energy recovery potential (McCarty et al., 2011). Microbial electrochemical technologies (METs), like microbial electrolysis cells (MECs), have shown great potential for recovering energy from wastewater that can offset treatment energy demands (Logan & Rabaey, 2012; Pant et al., 2012). In an MEC, a biotic anode, populated with

exoelectrogenic microbes that oxidize organic material and produce electrical current, is coupled with a hydrogen-evolving cathode (Liu et al., 2005; Rozendal et al., 2006). The reaction is not spontaneous and theoretically requires an additional applied potential of at least 0.11 V, although potentials greater than 0.5 V are typically required due to internal resistance and electrode overpotentials (Logan et al., 2008).

The organic material supplied to the anodic microbial community can come from a variety of waste sources. Domestic, swine farm, winery, food processing, industrial, landfill, and refinery effluents have been previously tested in MECs (Cusick et al., 2010; Liu et al., 2005; Lu et al., 2009; Ren et al., 2013; Tenca et al., 2013; Wagner et al., 2009). Solid biomass can also be used to generate hydrogen in a two-step process combining dark fermentation with electrohydrogenesis, with increased yields and conversion efficiency over dark fermentation alone (Lalaurette et al., 2009; Lu et al., 2009). Hydrogen and current generation varies between complex samples since organic concentration and composition can be significantly different (Cusick et al., 2010; Tenca et al., 2013). Chemical and biochemical oxygen demand are typical measures of organic strength and degradability in wastewater, but since these are based on either aerobic tests or complete chemical oxidation, and MECs are anaerobic systems, they do not directly relate to MEC performance (Ren et al., 2013). Therefore, direct measurements in MECs are necessary to evaluate performance with complex substrates.

High throughput mini MECs were recently developed as an inexpensive platform for conducting BES experiments (Call & Logan, 2011). Mini MECs have been previously used to evaluate treatment performance of industrial and domestic effluents (Ivanov et al., 2013; Ren et al., 2013), but the connection with performance in larger scale reactors has not been established. In this study, high-throughput mini MECs were compared with larger cube-type reactors used in

many other MEC tests, treating a variety of complex and ideal substrates to correlate performance between the systems. The goal was to evaluate the utility of mini MECs for screening wastewaters using a simpler and cheaper procedure than that of the larger cube MECs.

3.3 Materials and Methods

Effluent Samples

Industrial wastewater (IW) samples from a polymer and performance chemical production facility were collected and shipped in cooled containers overnight to Penn State. Three samples (IW-1, IW-2 and IW-3) were collected from different locations within the treatment operations at the facility. Sample IW-3 was collected just prior to effluent treatment after pH neutralization, IW-1 was collected just before pH neutralization, and IW-2 was collected at a point further upstream before all process effluents within the facility were combined.

Effluent from a dark fermentation process (FE), generated by *Clostridium thermocellum* fed with synthetic cellulose (Avicel, 5 g/L), was produced at the National Renewable Energy Lab in Golden, CO and shipped overnight to Penn State. Domestic wastewater (DW) samples were collected from the outlet of the primary clarifier at the Pennsylvania State University wastewater treatment facility (University Park, PA, USA). DW was evaluated in MECs and also served as a pre-acclimation substrate to enrich MEC anodes prior to tests with other samples. Acetate medium (AC), containing 1 g/L of sodium acetate dissolved in 50 mM PBS (2.45 g/L NaH_2PO_4, 4.58 g/L Na_2HPO_4) with additional nutrients added [0.31 g/L NH_4Cl, 0.13 g/L KCl, BOD nutrient buffer (Hach Co., Loveland, CO, USA)], was used as a positive control, as its composition does not vary. All samples were stored at 4°C prior to use in the experiments.

Reactor Construction

Mini MECs consisted of a 5 mL borosilicate serum bottle (Wheaton, Millville, NJ, USA) sealed with a butyl rubber stopper and aluminum crimp cap (Call & Logan, 2011) (Figure 3.1a). Anodes were made of 1.0 cm × 1.5 cm × 0.32 cm graphite blocks (Grade GM-10; GraphiteStore.com, Inc., Buffalo Grove, IL, USA) connected to titanium wire current collectors (0.032 gauge; Malin Co., Brookpark, OH, USA) that extended through the rubber stopper. Cathodes were made of stainless steel mesh (Type 304, 50 × 50 mesh size; McMaster-Carr, Elmhurst, IL, USA) cut to the same projected area as the anodes, and connected to stainless steel wire current collectors (0.032 gauge; Malin Co., Brookpark, OH, USA).

Cube MEC reactors were made from 4-cm long by 3-cm diameter cylindrical polycarbonate chambers (Lexan, 32 mL liquid volume) with a 1.6-cm diameter by 7-cm tall glass tube glued to the reactor top to provide gas headspace (Call & Logan, 2008) (Figure 3.1b). A carbon fiber brush (2.5-cm diameter by 2.5-cm length, Panex 35 polyacrylonitrile fiber; Zoltek, St. Louis, MO, USA) with a twisted core, titanium wire current collector, served as the anode. Brushes were heat treated at 450°C for 30 minutes before use to remove contaminants and create more favorable surface conditions for electrically active microbes (Feng et al., 2010). Cathodes were made of stainless steel mesh (Type 304, 50 × 50 mesh size; McMaster-Carr, Elmhurst, IL, USA) cut into 2 cm diameter discs with a total projected surface area of 12 cm^2 and 7 cm^2 exposed to the solution. A 0.5 mg/cm^2 platinum catalyst layer [10% (w/w) Pt on carbon black, Vulcan XC-72; Fuel Cell Store, College Station, TX, USA] was applied to the anode facing side of the cathodes using Nafion as a binder [5% solution (w/w), 33.33 $\mu L/cm^2$; Sigma Aldrich, St. Louis, MO, USA]. Gas bags (0.1 L capacity, Cali-5 bond, Calibrated Instruments Inc.,

Hawthorne, NY, USA) were connected to the headspace with plastic tubing and needles to collect additional gas and maintain atmospheric pressure in the headspace.

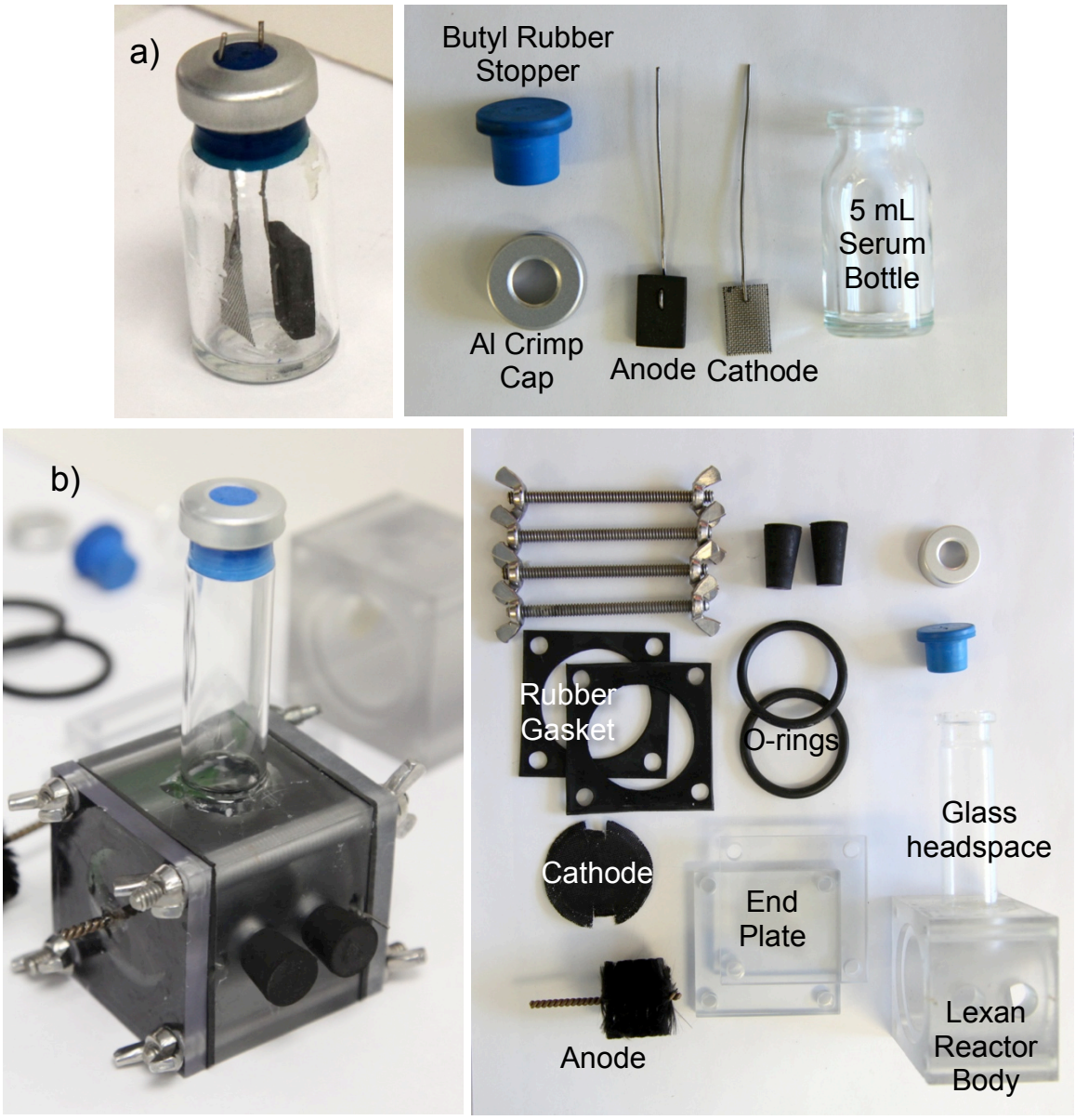

Figure 3.1 (a) High-throughput mini with a 5 mL liquid volume and (b) cube MEC reactors with a 32 mL liquid volume, with parts and components labeled.

Operation and Measurements

Mini reactors were operated in triplicate and cube MECs in duplicate in a 30°C controlled temperature room. Electrodes were connected to a programmable power supply (Model 3645A;

Circuit Specialists Inc., Mesa, AZ, USA) with an applied potential of 0.7 V for mini MECs and 0.9 V for cube MECs, consistent with previous tests (Cusick et al., 2010; Ren et al., 2013). A multimeter (Model 2700; Kiethley Instruments Inc., Cleveland, OH, USA) connected to a computer was used to record voltage measurements across a 10 Ω resistor placed in series between the positive terminal of the power supply and anode of each reactor. Current was calculated using Ohm's law ($U=IR$; A), where U (V) is the measured voltage, I (A) is current and R (Ω) is external resistance. Current density (j; A/m^2) was normalized to the projected cathode area and averaged over the time to reach 90% charge accumulation ($I_{avg\text{-}90}$), as previously described (Ivanov et al., 2013). The total charge recovered over a batch cycle was calculated by integrating the current over the cycle length ($C_T=\sum I\cdot\Delta t$; C). Coulombic efficiency (CE) was based on the total charge measured and change in chemical oxygen demand over a cycle (Ivanov et al., 2013).

Anode biofilms were pre-acclimated using DW as an inoculum and substrate, as this procedure has been shown to reduce startup time and improve subsequent performance (Ren et al., 2013). Mini MECs were fed DW until current profiles were repeatable for multiple cycles, and then switched to the individual samples. Cube MEC anodes were acclimated in microbial fuel cells (MFCs) fed DW before being transferred into clean MEC reactor bodies with new cathodes, and then switched to the individual samples. MFCs used for anode acclimation were 4-cm polycarbonate chambers, like the cube MEC bodies, with a 0.5 mg/cm platinum (10% w/w platinum on carbon black, Vulcan XC-72; Fuel Cell Store, College Station, TX, USA) catalyzed air cathode, prepared as previously described (Cheng et al., 2005). A 1000 Ω load was used as the external resistance during MFC operation.

Substrate was typically replaced in the MECs when the current in at least one reactor (two for mini MECs) in a replicate set decreased to less than 0.02 mA for the mini MECs and less than 0.2 mA for the cube MECs. After each batch cycle, effluent was removed, replaced with fresh substrate, and headspaces were sparged with anaerobic gas to remove oxygen and residual hydrogen and methane. Mini MECs were sparged for 2 minutes with an 80:20 N_2/CO_2 gas mixture and cube MECs were sparged for 20 minutes with ultra-high purity N_2 gas. Gas bags used with the cube MECs were sparged by filling with ultra-high purity N_2 gas and vacuuming empty three times in succession.

A gas chromatograph (GC) (Model 310; SRI Instruments, Torrance, CA, USA) with argon as a carrier gas and a 6-foot molecular sieve packed 5A column was used to measure gas concentrations of H_2, CH_4 and N_2 after cycles in cube MECs. A GC with helium as a carrier gas and a 6-foot Porapak Q column was used to measure CO_2 concentrations. Gas composition was determined by sampling the cube reactor headspace and gas bag with an airtight syringe (0.5 mL Gastight syringe; Hamilton Co., Reno, NV, USA). Gas quantity was determined using the known headspace volume (10 mL) and a gas bag method based on initial nitrogen gas concentrations (Ambler & Logan, 2011). Cathodic and overall gas conversion efficiencies for methane and hydrogen combined and hydrogen only were calculated as previously described (Wagner et al., 2009).

Influent and effluent chemical oxygen demand (COD) was measured using a standard chromic acid colorimetric method (Hach Co., Loveland, CO, USA). A three-day headspace biochemical oxygen demand ($HBOD_3$) test was used to determine influent and effluent biochemical oxygen demand (BOD) (Logan & Patnaik, 1997). The $HBOD_3$ test has been shown to provide results that are consistent with a conventional five day BOD test with less labor, time

and without sample dilution (Logan & Patnaik, 1997; Min et al., 2004). Primary clarifier effluent was used to provide an adequate microbial seed in all $HBOD_3$ vials and was added in a 50:50 ratio to the samples that were being tested. COD and $HBOD_3$ were measured once stable and repeatable current profiles were observed for consecutive batch cycles. Sample pH and conductivity were measured using a probe (Orion Versastar; Thermo Scientific, Waltham, MA, USA; SB90M5 SympHony; VWR, Radnor, PA, USA).

3.4 Results and Discussion

Wastewater Characteristics

Initial organic concentrations of the fermentation effluent (FE), industrial wastewater (IW), domestic wastewater (DW) and acetate (AC) samples were 450-7200 mg/L of COD (Figure 3.2). The raw fermentation effluent has a COD concentration of 7180 ± 100 mg/L and was diluted in 50 mM PBS to 1230 ± 70 mg/L before use in MEC tests to shorten the cycle length, as well as provide buffering capacity and solution conductivity. Although PBS addition is not realistic for larger applications, it was used to dilute the FE sample to provide an intermediate condition between the ideal (AC) and actual (IW, DW) wastewater samples by simulating the organic complexity of real wastewaters with the buffering capacity and conductivity of an ideal substrate. Sample IW-2 also had a high initial organic concentration of 6750 ± 100 mg/L of COD and produced little current and had long cycle times when added to MECs (Figure 3.3). Around day 40 of reactor operation, IW-2 was diluted in a NaCl solution to 1450 ± 40 mg/L of COD in an effort to reduce cycle time, while maintaining solution conductivity. NaCl solution was prepared to match the conductivity of the full-strength IW-2 sample, and mixed in a 3:1 ratio with IW-2.

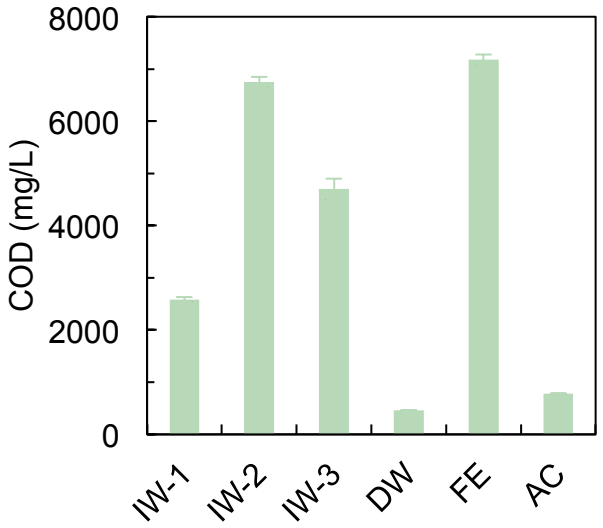

Figure 3.2 Initial COD concentration of samples as they were received prior to dilution (FE and IW-2) and pH adjustments (IW-1 and IW-2).

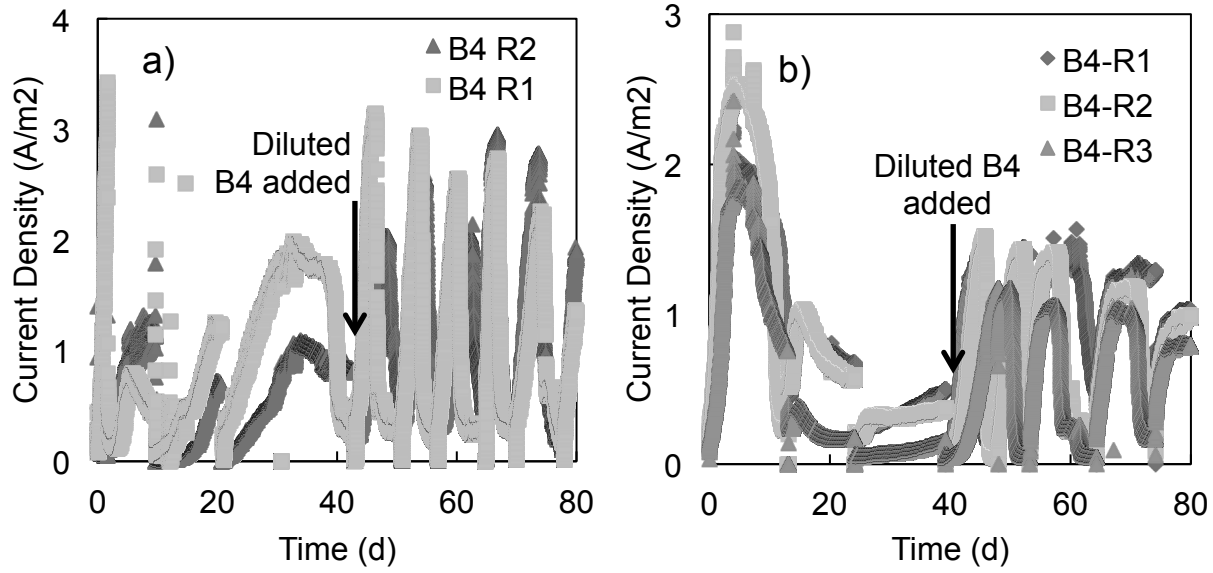

Figure 3.3 Current profiles for (a) cube and (b) mini MECs fed sample IW-2. Both reactors were switched from the full strength to diluted IW-2 samples around day 40, after exhibiting low and unstable current generation.

Initial pH for IW-3, FE, DW and AC samples ranged from 7.0-8.2. Samples IW-1 and IW-2 had an initial pH below 5.5 (Figure 3.4a), which was outside the optimal range for exoelectrogenic microbes (He et al., 2008). To ensure that MEC performance was not inhibited

by low pH, IW-1 and IW-2 were neutralized to pH 7.3 with 0.3 M NaOH before testing in MEC reactors. Solution conductivity, measured after pH and organic concentration adjustments, ranged between 0.7-1.9 mS/cm for the non-buffered wastewater samples (IW-1, IW-2, IW-3, DW) and 3.7-4.0 mS/cm for the FE and AC samples (Figure 3.4b).

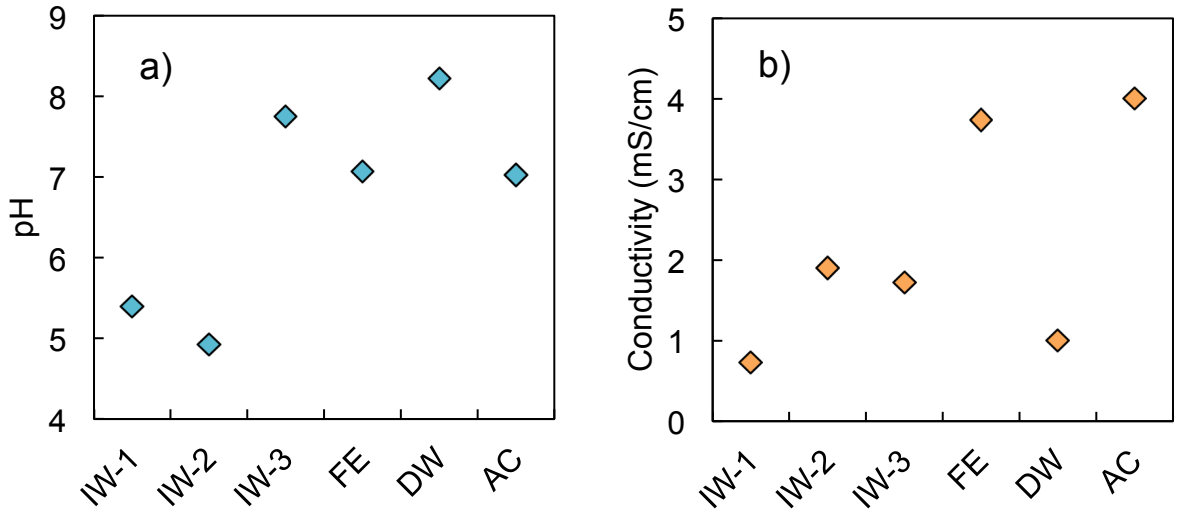

Figure 3.4 (a) Initial sample pH, measured prior to neutralization for IW-1 and IW-2 and (b) conductivity of samples as they were used in MEC reactors.

Influent COD and $HBOD_3$ concentrations of the samples as they were used in MECs ranged between 450-4500 mg/L of COD and 230-790 mg/L of $HBOD_3$ (Figure 3.5). Of the six samples tested, the three industrial wastewater samples (IW-1, IW-2, IW-3) had the highest influent COD concentrations, all exceeding 1400 mg/L. The COD/$HBOD_3$ ratio provided insight into the aerobic biological degradability of the organic material in each sample, with lower values indicative of a more easily degradable substrate. IW-1 and IW-3 had COD/$HBOD_3$ ratios greater than 5, meaning a significant portion of the COD was not readily degradable under aerobic conditions within 3 days. AC, which contained organic matter that was readily degraded, had a COD/BOD_3 of 1.3, and all other samples had ratios below 3.

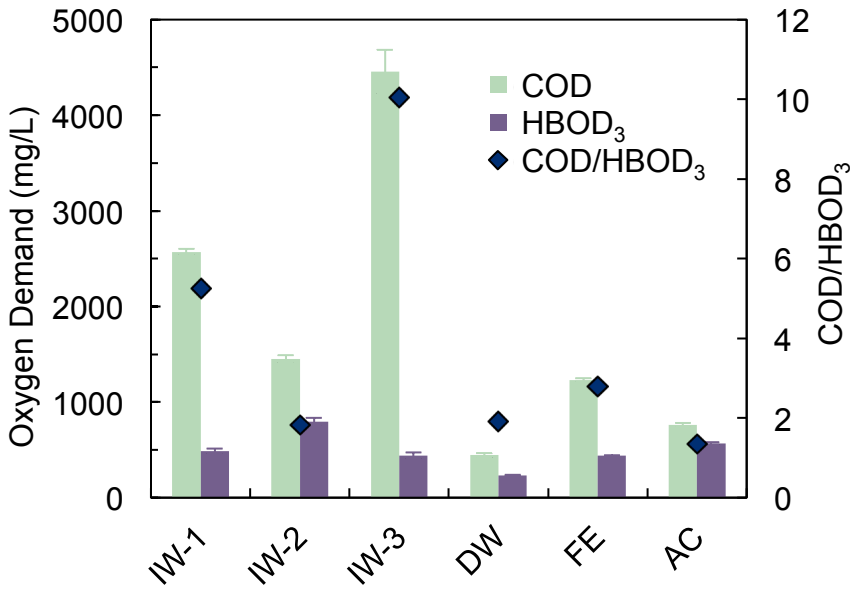

Figure 3.5 Influent COD and $HBOD_3$ concentrations and $COD/HBOD_3$ ratio prior to MEC treatment, measured after pH adjustments to IW-1 and IW-3 and dilution of IW-2 and FE samples.

COD Removal and Coulombic Efficiency

Between 66-92% of the influent COD was removed in mini MECs, and 74-90% in cube MECs over a fed-batch cycle for each sample (Figure 3.6a). Average cycle length varied between samples and was generally 2-11 days (Figure 3.6b). Effluent COD concentrations in the IW samples ranged from 360-780 mg/L, which was higher than the rest of the samples tested (Figures 3.6c). IW-2 and IW-3 had significantly lower effluent COD concentrations in cube MECs than mini MECs, while FE and AC were significantly lower in mini MECs (T-test, $p<0.01$). IW-1 and DW effluent COD concentrations were not significantly different between mini and cube MECs. The difference in COD removal between the mini and cube MECs with the IW-2 and IW-3 samples could have been a result of gas buildup in the headspace of the mini MECs, which could have inhibited gas production and COD removal compared to cube MECs that had gas-bags to alleviate headspace pressure. The AC sample had the lowest effluent COD

concentration and highest overall removal, which was expected because acetate is readily degraded by exoelectrogens. IW-3 had the highest influent COD concentration and COD/$HBOD_3$ ratio, but had the second highest COD removal of 82 ± 5% in the mini and 86 ± 2% in the cube MECs.

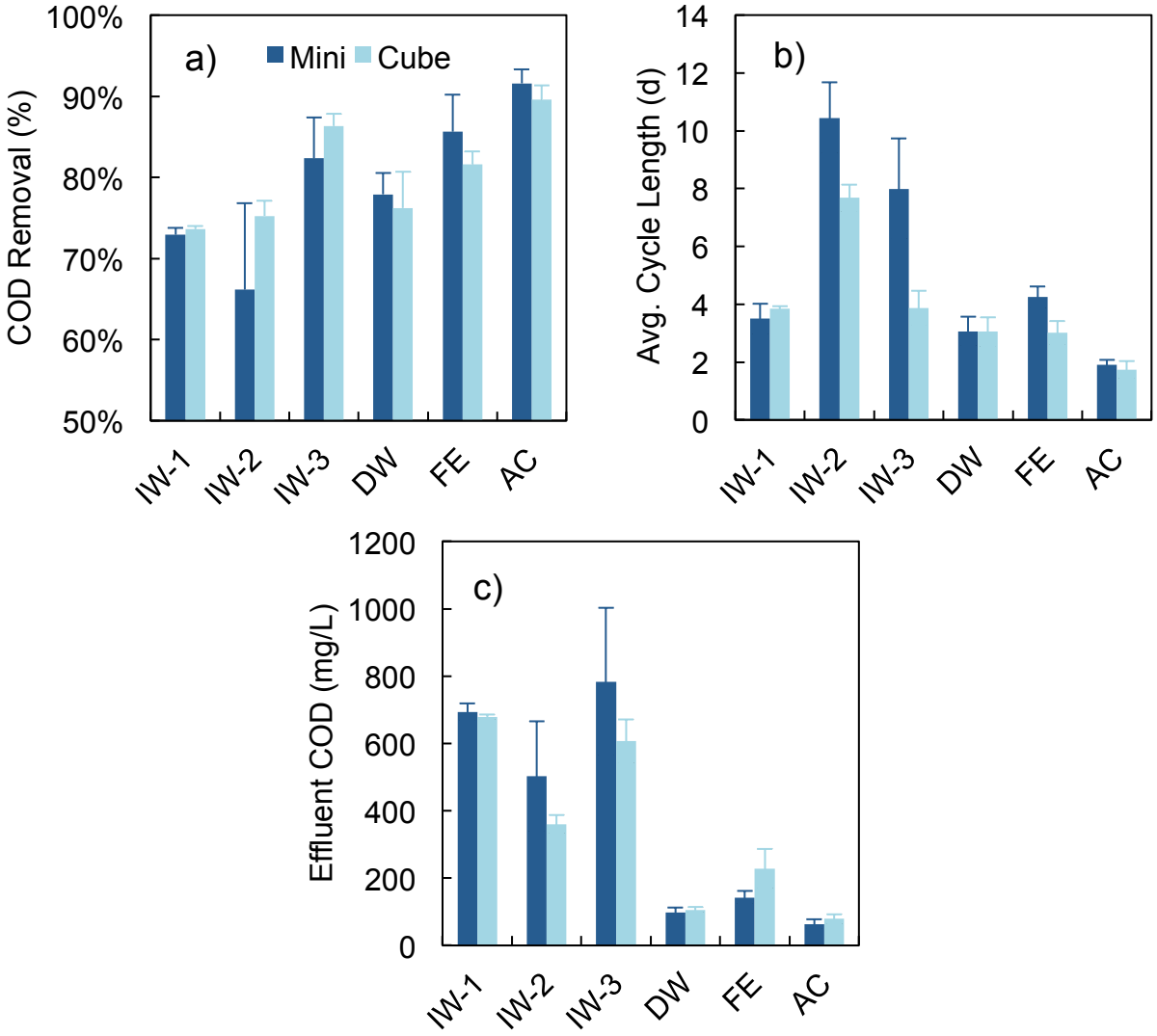

Figure 3.6 (a) COD removal, (b) average cycle length, and (c) effluent COD concentration for mini and cube MECs fed each sample. Results were averaged over multiple cycles.

Average coulombic efficiencies (CE) varied significantly between samples, ranging from 23-169% in the mini MECs and 18-132% in the cube MECs (Figure 3.7a). Samples IW-1 and IW-3 had coulombic efficiencies of 38 ± 3% and 23 ± 6% for mini MECs and 41 ± 2% and 18 ±

3% for cube MECs. A low CE indicates that a large portion of COD removal was not due to current generation but instead a result of reactions other than anode respiration. Open circuit cycles confirmed significant COD removal in the absence of current generation for the IW-1 and IW-3 samples (Figure 3.7b). The CEs for IW-2 in the mini and cube MECs exceeded 100% (169 ± 60% and 115 ± 11%), suggesting that microbial hydrogen oxidation at the anode was contributing to the measured current density. Oxidation of hydrogen evolved at the cathode by exoelectrogenic microbes on the anode, also known as hydrogen cycling, is an issue in single chamber MECs since there is no separation between the anode and cathode (Lee & Rittmann, 2010). The average CE for the DW sample was also greater than 100% in cube MECs (132 ± 19%), but was 89 ± 7% in the mini MECs. Current was unstable at the end of later DW fed cube MEC cycles, while current in mini MECs fed DW was stable (Figure 3.8). Erratic current in the cube MECs fed DW likely contributed to the difference in CEs between mini and cube reactors, although it is not known why this was only observed with DW fed MECs.

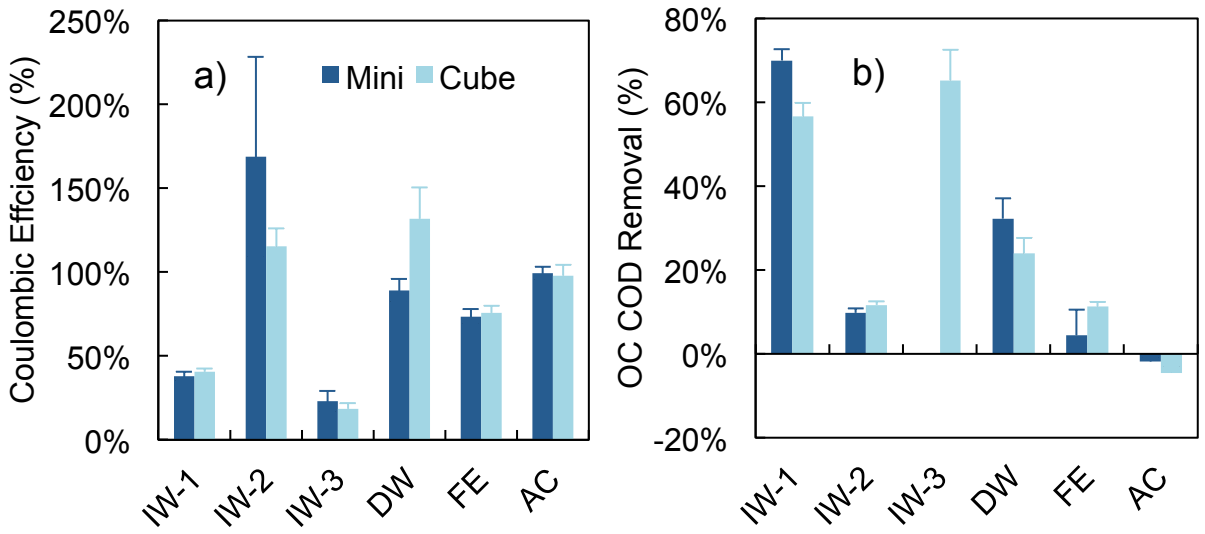

Figure 3.7 (a) Coulombic efficiency (CE), which is the ratio of electron equivalents measured as current to those removed as COD, for MECs fed each sample and (b) COD removal during open circuit operation when electrodes were disconnected and no current was generated.

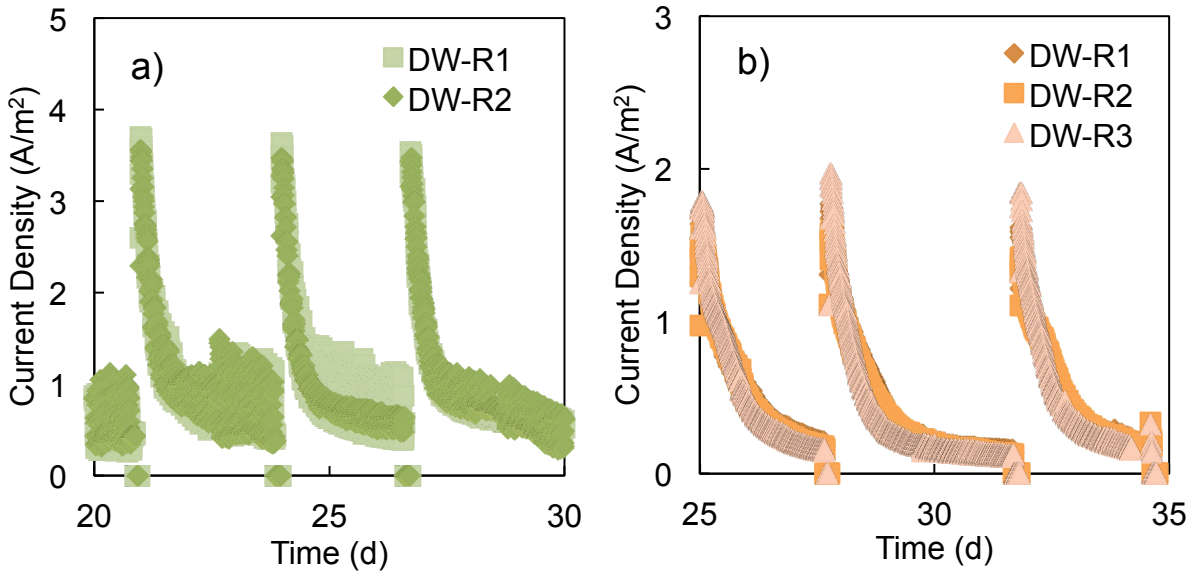

Figure 3.8 Representative current density profiles for (a) cube and (b) mini MECs fed DW sample.

Current Generation

Average current densities, calculated over the time to 90% charge accumulation ($I_{avg\text{-}90}$), varied between wastewater samples and reactor types. $I_{avg\text{-}90}$ current densities were higher in cube MECs for all samples (except IW-1), which could be partially attributed to the platinum catalyst layer used on the cathodes in the cube MECs (Figure 3.9a). The time to achieve 90% charge accumulation (t_{90}) in mini MECs was also longer than those in the cubes for IW-2, IW-3, and FE, which could have contributed to the difference in average current density between mini and cube reactors (Figure 3.9b). AC and FE samples produced the highest current densities of the samples tested in both mini and cube reactors. IW-1, IW-2, and IW-3 average current densities in both mini and cube MECs were significantly less than AC, but still greater than DW.

The energy required for organic treatment, which was based on the additional energy added to the MECs through the external power supply, was highest in MECs fed IW-2 and DW (Figure 3.10a). The high treatment energy observed in these samples was likely due to hydrogen

cycling, which generates current without oxidizing organic material. The energy requirements for the IW-1, IW-3, FE, and AC samples varied between substrates, but were generally slightly higher in cube MECs due to the difference in applied potential. Total charge recovered, normalized to the reactor volume, also varied between individual samples, but was reasonably consistent between cube and mini MECs fed IW-1, IW-2, FE, and AC (Figure 3.10b). There was no significant difference (T-test, $p>0.03$) in total charge between mini and cube MECs for samples IW-2, IW-3, FE, and AC.

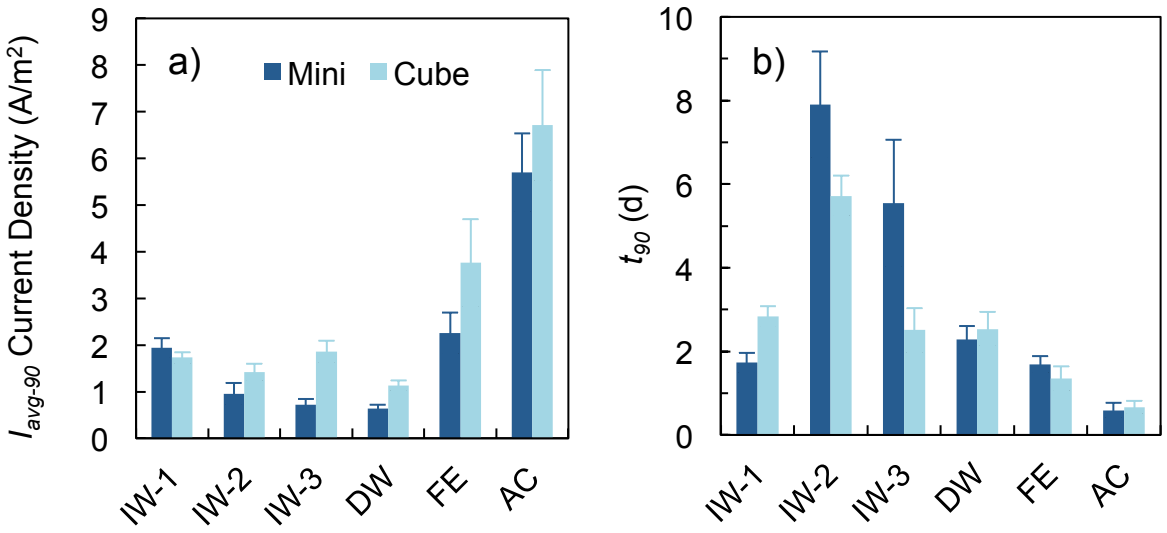

Figure 3.9 (a) Current density averaged over the time to 90% charge accumulation ($I_{avg\text{-}90}$) and (b) time to 90% charge accumulation (t_{90}) in mini and cube MECs.

Charge accumulation, which is the coulombs transferred through the circuit at a given time expressed as a percentage of the total coulombs recovered over the cycle, was consistent between reactor types with ideal substrates, but different for the real wastewater samples. AC and FE, which were buffered and contained easily degradable organic material, exhibited very similar profiles in both mini and cube MECs, with a nearly linear initial period followed by a plateau (Figure 3.11). DW fed reactors showed reasonable agreement between charge accumulation profiles, but the charge profiles did not exhibit a pronounced plateau like those

observed with AC and FE solutions. Charge accumulated faster in the cube MECs fed IW-2 and IW-3 than in the mini MECs, but the opposite was observed for IW-1 with faster charge accumulation in the mini MECs. Both reactors fed IW-2 and the mini MECs fed IW-3 also showed a non-linear response at the beginning of cycles, indicating that there was some lag time before the maximum charge accumulation rate was obtained. This would suggest that the organic material in the IW-2 and IW-3 samples was degraded at a slower rate than other samples. The different profiles for the mini and cube MECs with complex substrates indicated that performance differences between reactor types are primarily due to the substrate, but the reactor design is also a factor.

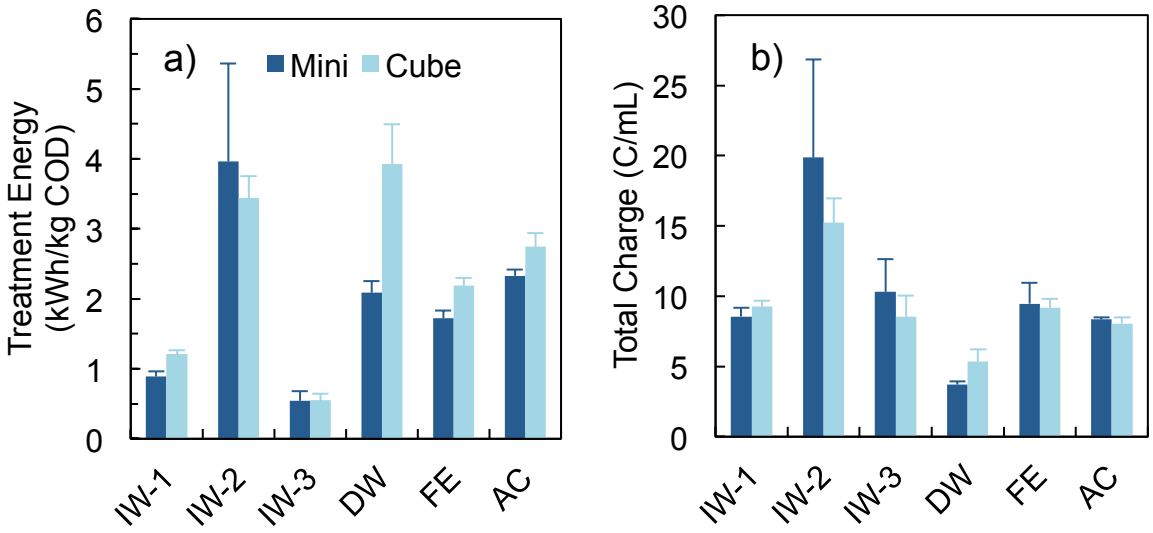

Figure 3.10 (a) Treatment energy based on measured current, applied potential, and COD removal and (b) total charge recovered (normalized to the reactor liquid volume) over batch cycles with various substrates in mini and cube MECs.

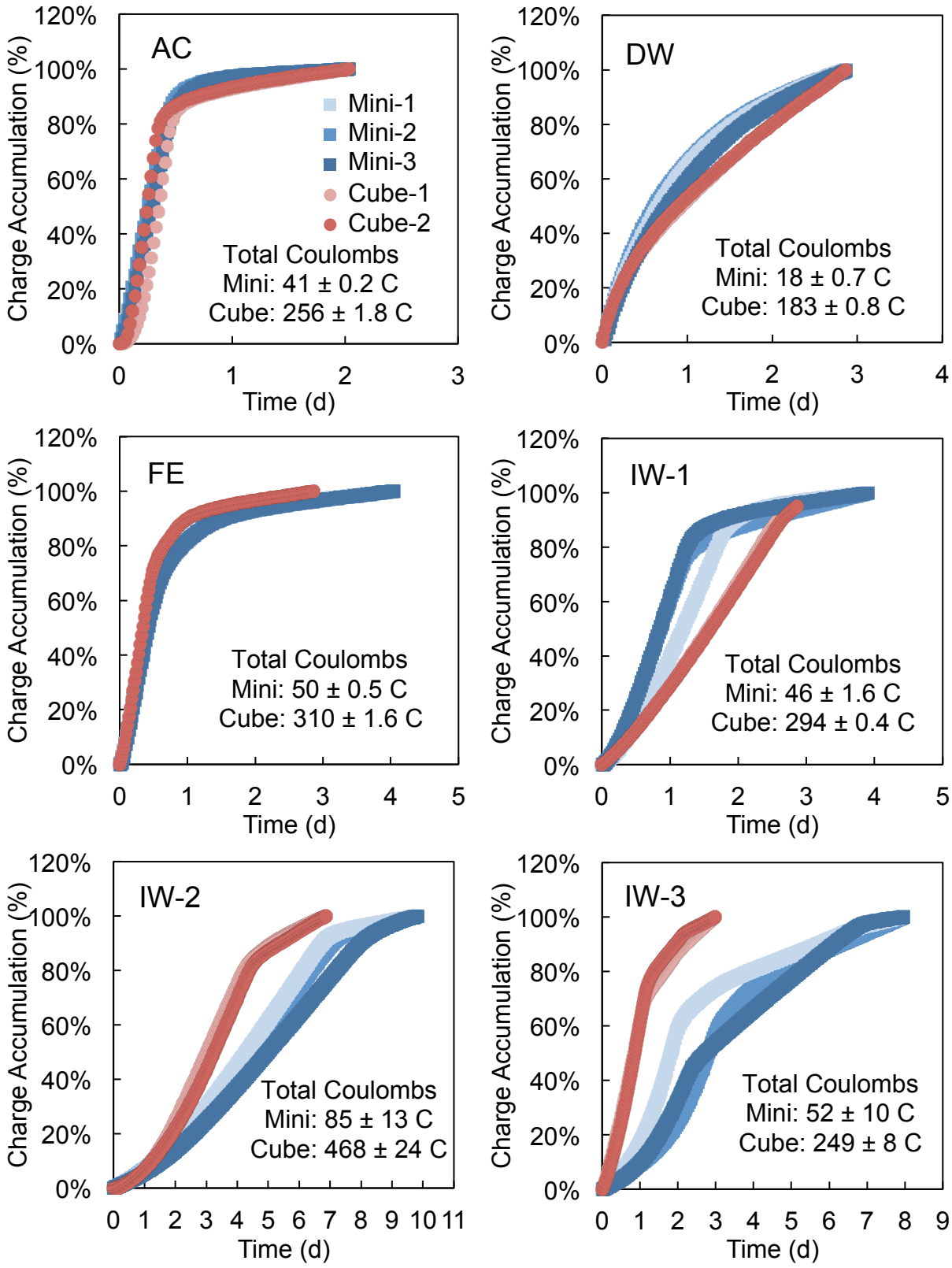

Figure 3.11 Charge accumulation in triplicate mini and duplicate cube MEC reactors as a percentage of the total coulombs measured over a cycle for each substrate, with average total coulombs for each reactor set shown.

Comparison of Mini and Cube MEC Treatment Performance

Treatment performance and efficiency in mini and cube MECs was examined using a linear regression to determine if there were significant performance correlations between the two reactor designs. Total COD removal and CE were significantly (R^2>0.99, p<0.01) related between the reactor types, with slopes very close to 1, indicating that treatment performance was consistent between mini and cube MECs (Figure 3.12). IW-2 and DW samples were not included in the CE linear regression because they both had values greater than 100%, likely due to hydrogen cycling between the electrodes. Total COD removal generally varied by less than 10% between reactors, and CE varied by less than 20% (excluding IW-2 and DW samples) (Figure S.1a).

$I_{avg\text{-}90}$ current density and effluent COD were also significantly related (R^2>0.92, p<0.01) between the reactor types (Figure 3.13). Average current density for AC was significantly higher in both mini and cube MECs than the FE, IW, and DW samples. When AC was not included in the regression, the relationship for average current density was no longer significant (R^2<0.6, p>0.12), indicating that the trend was disproportionally influenced by the AC values. Effluent COD for the IW samples was lower in the cube MECs than the mini MECs, while effluent COD for the DW, AC, and FE samples was lower in the mini MECs. The values for effluent COD concentration were reasonably well distributed, but the residuals showed a trend with organic strength, indicating that COD removal efficiency in the mini MECs may have been influenced by the organic concentration of the substrate (Figure S.1b). The difference in effluent COD concentration between the mini and cube MECs could also be due to other characteristics of the wastewater samples since only a small number of samples were examined in this study, and because the IW samples all came from the same facility.

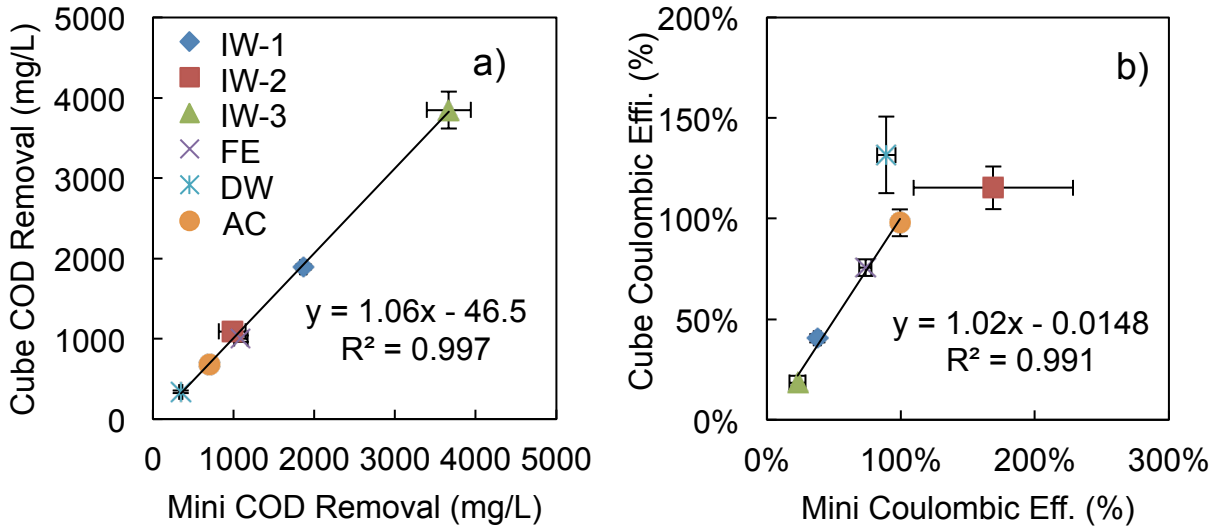

Figure 3.12 (a) Linear comparison of total COD change and (b) coulombic efficiency between MEC reactor types. CE with samples IW-2 and DW exceeded 100%, indicating that hydrogen cycling was occurring, and they were omitted from the coulombic efficiency linear fit.

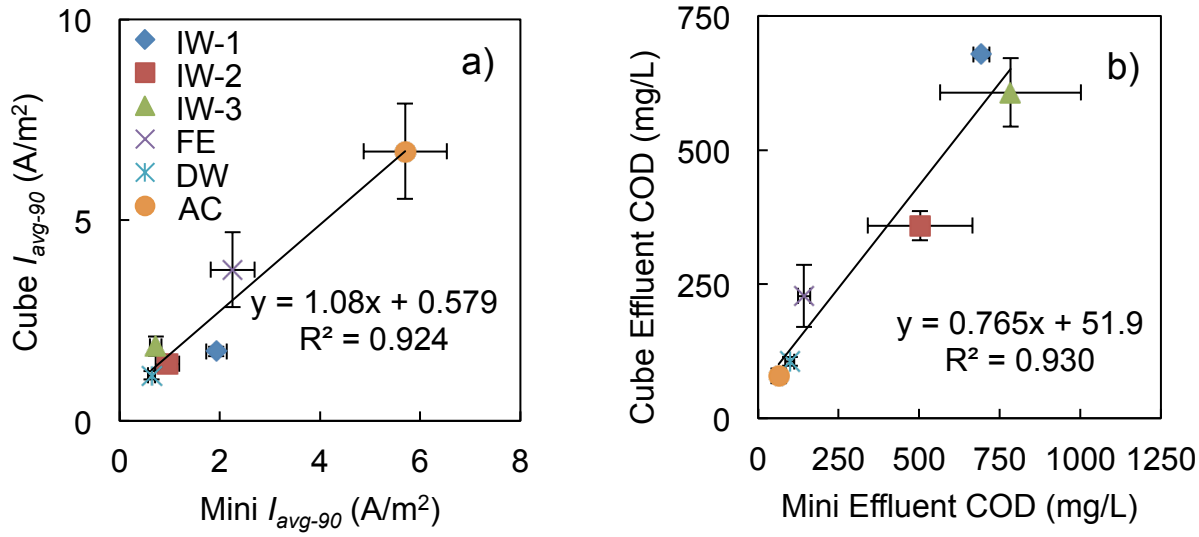

Figure 3.13 (a) Linear relationship between mini and cube MEC I_{avg-90} current density and (b) effluent COD concentration.

Gas Recovery in Cube MECs

Measurable concentrations of hydrogen and methane were observed in cube MECs with each individual sample. Total measured biogas volume was greatest for the IW samples, although more methane than hydrogen gas was measured with IW-1 and IW-3 (Figure 3.14a). Cube MECs with IW-2 generated 24 ± 10 mL of hydrogen over batch cycles, which was similar to AC (22 ± 3 mL), but cycle time was significantly longer at nearly 8 days for IW-2 versus ~2 days for AC. There was a significant (R^2=0.90, p<0.01) relationship between $HBOD_3$ removal and volume of hydrogen gas recovered, but the measured gas volume was inconsistent over multiple cycles with some samples (Figure 3.14b). Measured hydrogen gas volumes for samples IW-1, IW-2, and DW were the most inconsistent over time, as exhibited by large standard deviations relative to the average.

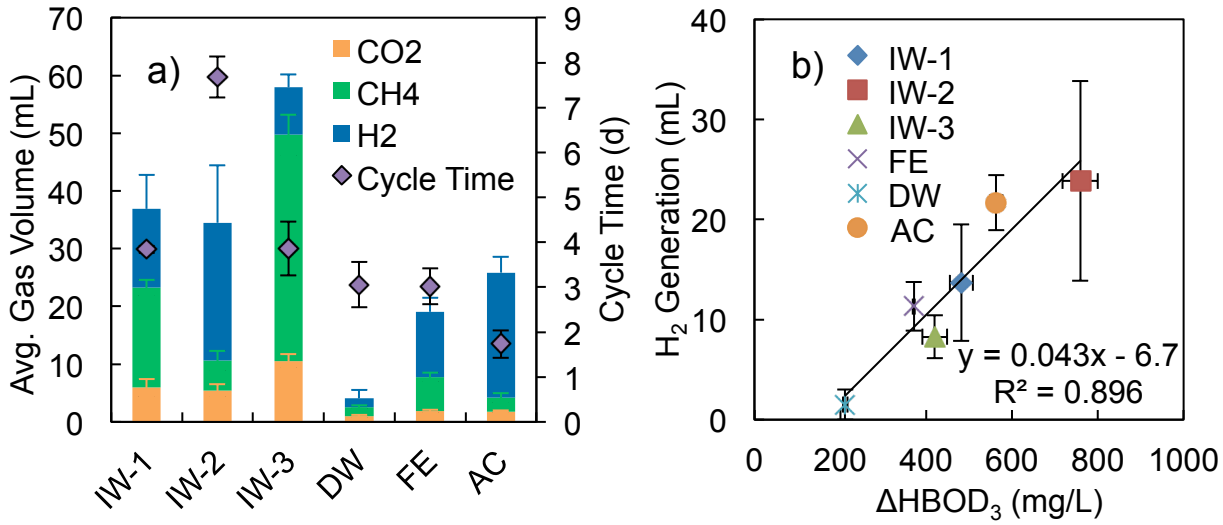

Figure 3.14 (a) Cube MEC average gas composition and cycle time and (b) linear relationship between change in $HBOD_3$ and hydrogen gas generation.

The energy contained in the recovered hydrogen and methane gas, based on the heat of combustion (ΔH_{H2} = 285.8 kJ/mol, ΔH_{CH4} = 891 kJ/mol), exceeded the energy that was added through the power supply for all samples, except DW (Figure 3.15). When the efficiency was

computed using just the recovered hydrogen, only the MECS fed AC exceeded 100%, although this does not account for hydrogenotrophic methanogenesis, which would reduce the hydrogen yield. The energy efficiency also does not account for the energy in the substrate. Using the heat of combustion to estimate the energy content of the gas also assumes that the gas is converted into energy, but hydrogen is a valuable product that can be used for other processes.

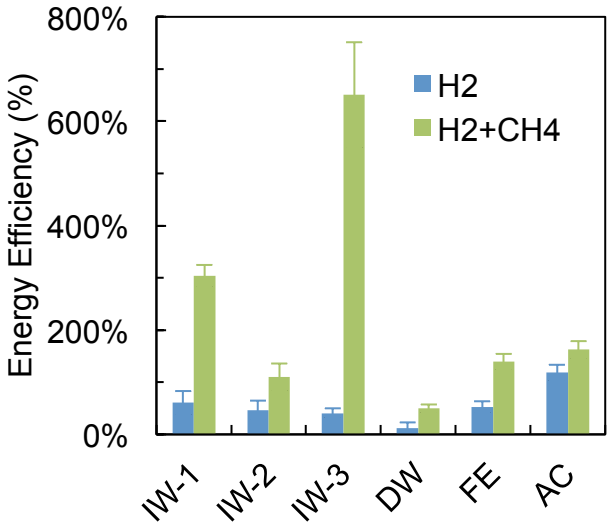

Figure 3.15 Energy efficiency for cube MECs with each sample based on the energy content of the gas recovered and the energy added through the external power supply. The efficiency was calculated using only recovered hydrogen and combined hydrogen and methane, using the heat of combustion to calculate the energy contained in the gas.

Cathodic hydrogen recovery ($r_{H2,\ cat}$) represents the fraction of current that was recovered as hydrogen gas, while the overall hydrogen recovery ($r_{H2,\ COD}$) is based on the total COD converted to hydrogen. Cathodic hydrogen recovery was less than 40% for all samples except AC, which had a 67% hydrogen gas recovery (Figure 3.16a). The combined cathodic recovery for both hydrogen and methane ($r_{H2+CH4,\ cat}$) was 96% for the FE and AC samples, indicating that nearly all the generated current went into gas production (Figure 3.16b). Most of the methane generated with IW-1 and IW-3 was likely from direct methanogenesis of the substrate since combined cathodic recoveries were well over 100%, and substantial methane production

occurred in open circuit controls (Figure 3.17). Hydrogen gas cycling between the anode and cathode for the DW and IW-2 samples, which results in current generation without net gas production, most likely contributed to low cathodic gas recoveries of 37% and 74%. Nearly all the COD removed in cube MECs for IW and AC samples was converted to gas, with an overall gas recovery ($r_{H2+CH4,\ COD}$) of 84-94% (Figure 3.16b). Overall gas recovery was 72% for FE and only 48% for DW. Alternative electron acceptors, like O_2, NO_3^-, and SO_4^{2-}, could have contributed to the low overall gas recoveries for these samples, but these species were not analyzed in this study as the focus was on treatment efficiency.

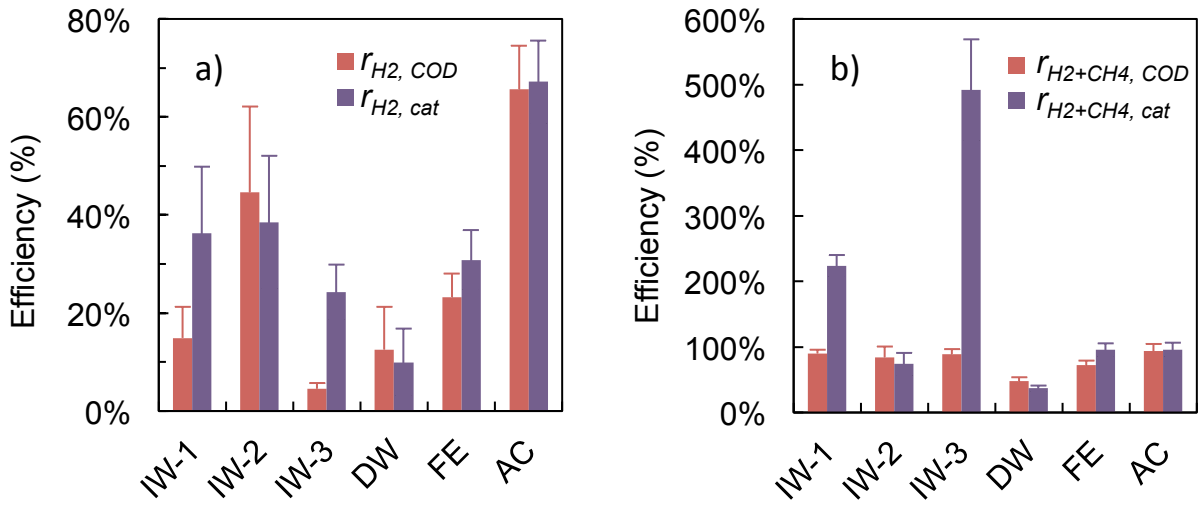

Figure 3.16 (a) Cathodic ($r_{H2,cat}$) and overall ($r_{H2,COD}$) recovery of hydrogen based on COD and current and (b) cathodic ($r_{H2+CH4,cat}$) and overall ($r_{H2+CH4,COD}$) recovery of hydrogen and methane based on COD and current.

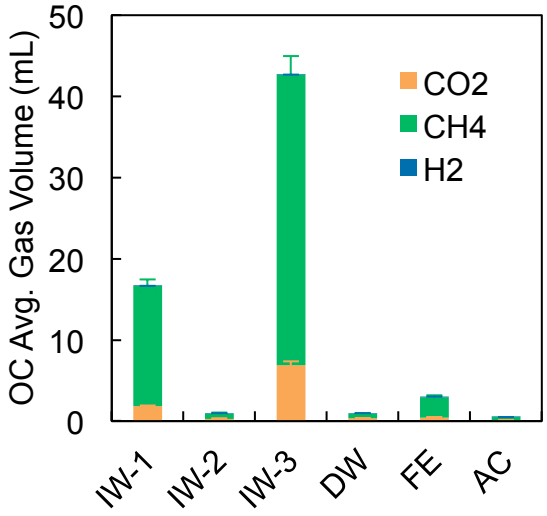

Figure 3.17 Open circuit gas production and COD removal in cube MECs, showing substantial methane generation with IW-1 and IW-3.

Current, COD removal and gas production in an MEC should be proportional to each other if alternate electron acceptors are not available for substrate removal. This was observed here for MECs fed AC, as nearly all of the electrons generated from organic oxidation at the anode were measured as current through the external circuit and recovered as gas, with coulombic efficiencies, cathodic gas recoveries, and overall gas recoveries between 94-99%. Results for MECs fed domestic and industrial wastewater were less consistent. In MECs fed IW-1 and IW-3, electrons recovered in the gas exceeded those measured as current due to direct substrate methanogenesis. Hydrogen cycling in MECs fed IW-2 and DW resulted in current generation that did not translate to gas production. Therefore, while COD removal was consistent with current generation and gas production in MECs fed ideal substrates, real wastewaters were more complex and the relationship between these performance parameters was less predictable.

3.5 Conclusions

Organic removal and treatment efficiency in high-throughput mini MECs corresponded well to performance in larger cube reactors despite differences in reactor materials, configurations, and applied potentials. COD removal and coulombic efficiency for individual samples were significantly correlated between reactor types. Average current density was related between mini and cube MECs, although the cathode catalyst layer and higher applied potential contributed to generally higher current density in cube MECs. COD removal, current generation and measured gas in cube and mini MECs were significantly different between individual samples. Mini and cube MECs fed buffered samples were generally more consistent and produced more current than domestic and industrial wastewater fed reactors, but the industrial effluents tested in this study generated more gas and current than a domestic wastewater sample. Current recovery was similar between mini and cube MECs fed well-buffered samples, but significantly different with industrial effluents. Although current generation was not identical to cube MECs with industrial wastewater samples, high-throughput mini MECs were useful for evaluating substrate performance and provide a simpler, cheaper procedure for screening wastewater samples.

4. ANODE ACCLIMATION METHODS AND THEIR IMPACT ON MICROBIAL ELECTROLYSIS CELLS TREATING FERMENTATION EFFLUENT

4.1 Abstract

Mini and cube microbial electrolysis cells (MECs) were acclimated to wastewater or acetate to develop an electrically active biofilm prior to tests using fermentation effluent, which slightly improved COD removal (3-5%) compared to non-acclimated reactors. Treatment performance and current generation in MECs acclimated to domestic wastewater was as good as, or better than, acetate acclimated reactors, indicating that acetate addition may not be necessary in developing electrically active biofilms for treating fermentation effluent. Although acclimating reactors prior to fermentation effluent tests improved treatment, differences in performance due to reactor types (mini versus cube MECs) were more significant than acclimation method. These results indicates that MEC materials and configuration have a greater influence on treatment and current generation than biofilm acclimation method, and that acclimation using domestic wastewater and fermentation effluent, or domestic wastewater alone, are suitable methods for MEC acclimation to achieve treatment of fermentation effluent.

4.2 Introduction

Hydrogen gas is widely used for industrial purposes, including gas and oil refining, food processing and fertilizer production, and is primarily generated from fossil fuels (EIA, 2008; Logan, 2004). Electrolysis is a viable alternative to fossil fuel based hydrogen production methods, but since it requires electricity, it can still be indirectly dependent on fossil fuels (Keith

& Farrell, 2003). Biological hydrogen production methods, including photolysis and fermentation, can be used to generate hydrogen from organic waste streams, such as biomass and wastewater (Angenent et al., 2004; Lee et al., 2010; Levin et al., 2004). Using a dark fermentation process, one mole of glucose can stoichiometrically produce 12 moles of hydrogen, but maximum yields of 2-3 mol H_2/mol glucose equivalent are typically observed (Angenent et al., 2004; Datar et al., 2007; Lalaurette et al., 2009; Levin et al., 2006; Lu et al., 2009; Valdez-Vazquez & Poggi-Varaldo, 2009). The dark fermentation effluent is rich in organic acids, ethanol, and protein that cannot be further fermented to produce hydrogen, limiting biomass conversion efficiencies (Levin et al., 2006; Magnusson et al., 2008; Wang et al., 2011).

More hydrogen could be extracted from dark fermentation effluent using electrohydrogenesis, another biologically dependent method of hydrogen production. In a microbial electrolysis cell (MEC), exoelectrogenic microbes generate an electrical current by oxidizing organic matter. Electrons travel through an external circuit and are recombined at the cathode with protons to form hydrogen gas (Liu et al., 2005; Rozendal et al., 2006). MECs require a source of electrical power since the overall reaction is not thermodynamically favorable, although much less voltage is needed than that used for water electrolysis (Liu et al., 2005). Unlike dark fermentation, MECs can be used to almost completely oxidize organic material into CO_2, which makes them optimally suited to treat the soluble organic products generated during dark fermentation (Lee et al., 2010). MEC treatment combined with dark fermentation has increased hydrogen yields to nearly 10 mol H_2/mol glucose equivalent, which is significantly greater than typical yields of 2-3 mol H_2/mol glucose using dark fermentation alone (Lalaurette et al., 2009).

Exoelectrogenic microbes are prevalent in nature, and electrically active biofilms can be developed using a wide range of inoculum sources (Logan, 2009). A number of studies have investigated the microbial communities that develop in MECs with different inoculum sources and found that *Geobacter* species are typically prevalent, regardless of the microbial diversity of the inoculum (Call et al., 2009; Cusick et al., 2010). Effluents rich in microorganisms and organic matter, like domestic wastewater, can be used as both substrate and inoculum (Cusick et al., 2010). For industrial wastewater samples with limited biological activity, developing MEC anode biofilms with domestic wastewater and switching to the industrial effluent has been shown to improve treatment performance, compared with acclimation to a mixture of industrial and domestic wastewater (Ivanov et al., 2013; Ren et al., 2013). Acclimating anodes to acetate or synthetic wastewater in MFCs prior to use in MECs is another method to enrich an electrically active biofilm, but chemical addition can be expensive and starting anodes in MFCs is not practical for larger applications (Cusick et al., 2011; Cusick et al., 2010; Lalaurette et al., 2009).

A number of methods have been used to successfully develop electrically active biofilms in MECs, but the influence of acclimation method on current generation, organic removal, and gas production in MECs treating fermentation effluent has not been examined. To investigate how acclimation influences MEC performance, high-throughput mini MECs were first acclimated to domestic wastewater or acetate, and then switched to fermentation effluent. Cube MEC anodes were also first acclimated to domestic wastewater in MFCs, and transferred into MECs fed fermentation effluent. Mini and cube MECs were also started on fermentation effluent with domestic wastewater inoculum and no anode pre-acclimation. Performance was evaluated based on COD treatment, protein removal and current generation with mini and cube MECs, as well as gas recovery with cube MECs.

4.3 Materials and Methods

Fermentation Effluent and Acclimation Substrates

Fermentation effluent was produced in continuous cultures using *Clostridium thermocellum* fed 1191 medium containing synthetic cellulose (Avicel, 5 g/L) at the National Renewable Energy Lab in Golden, CO (Levin et al., 2006). Effluent samples were stored on ice and shipped overnight to Penn State. Raw fermentation effluent had a COD concentration of 7180±100 mg/L, conductivity of 4.5 mS/cm and a pH of 7.1. The raw fermentation effluent was diluted in 50 mM PBS to 1230±70 mg/L of COD with a pH of 7.1 and conductivity of 3.7 mS/cm before use in MEC reactors. Domestic wastewater, used as inoculum and substrate for pre-acclimation, was collected from the outlet of the primary clarifier at the Penn State University wastewater treatment plant (University Park, PA, USA). New domestic wastewater samples were collected at least every 2 weeks. Acetate medium used during acclimation consisted of 1 g/L of sodium acetate dissolved in 50 mM phosphate buffer solution (PBS; 2.45 g/L NaH_2PO_4, 4.58 g/L Na_2HPO_4) with additional nutrients added [0.31 g/L NH_4Cl, 0.13 g/L KCl, trace vitamins and minerals (Balch et al., 1979)].

Reactor Construction

High-throughput mini MECs were constructed as previously described (Call & Logan, 2011) using 5 mL glass serum bottles (Wheaton, Millville, NJ, USA) as the reactor body, graphite plate anodes (1.5 × 1 × 0.32 cm, Grade GM-10; GraphiteStore.com, Inc.) and stainless steel mesh cathodes (1.5 × 1 cm, Type 304, 50 ×50 mesh size; McMaster-Carr, Elmhurst, IL, USA). Graphite anodes were connected to titanium wire current collectors, while cathodes were connected to stainless steel wires (0.032 gauge; Malin Co., Brookpark, OH, USA). A butyl

rubber stopper and aluminum crimp cap were used to seal each reactor, with the current collecting wires extending through the stopper so they could be connected to an external circuit. Mini MECs were operated in triplicate for each condition.

Cube MEC reactors were constructed from a 4-cm long by 3-cm diameter cylindrical polycarbonate reactor chamber (Lexan, 32 mL liquid volume) with 1.6-cm diameter by 7-cm tall glass tube headspace (Call & Logan, 2008). The anodes were carbon fiber brushes with twisted titanium wire current collectors (2.5-cm diameter × 2.5-cm length, Panex 35 polyacrylonitrile fiber; Zoltek, St. Louis, MO, USA) that were heat treated at 450°C for 30 minutes before use to remove contaminants and create more favorable surface conditions for electrically active microbes (Feng et al., 2010). Cathodes were constructed form stainless steel mesh (Type 304, 50 × 50 mesh size; McMaster-Carr, Elmhurst, IL, USA) with a catalyst layer, consisting of 0.5 mg/cm^2 platinum [10% (w/w) on carbon black, Vulcan XC-72; Fuel Cell Store, College Station, TX, USA] with Nafion as a binder [5% solution (w/w), 33.33 μL/cm^2; Sigma Aldrich, St. Louis, MO, USA], painted onto the liquid facing side of the cathode. Gas bags (0.1 L capacity, Cali-5 bond, Calibrated Instruments Inc., Hawthorne, NY, USA) were connected to the headspace with plastic tubing and needles to collect additional gas and maintain atmospheric pressure in the headspace. Duplicate cube MECs were operated for each condition.

Anode Pre-acclimation

Mini MECs were operated with either domestic wastewater (M-WW) or acetate media (M-AC) as substrate to enrich the anodic biofilm prior to tests with fermentation effluent, and were compared to mini MECs that were only fed fermentation effluent and did not have a pre-developed anodic biofilm (M-FE+WW). Inoculum was added to the M-AC and M-FE+WW reactors in a 1:1 ratio with substrate and was omitted once reactors reached 0.5 mA. Domestic

wastewater served as inoculum and media for M-WW reactors. M-AC reactors were fed acetate media, and inoculated with MFC effluent. M-FE+WW reactors were not pre-acclimated and were started directly with fermentation effluent substrate and domestic wastewater inoculum.

Cube MEC anodes were pre-acclimated in microbial fuel cells (MFCs) with domestic wastewater as substrate and inoculum. MFCs used for anode acclimation were 4-cm polycarbonate chambers with the same dimensions as the cube MECs and a 0.5 mg/cm^2 platinum (10% w/w Pt on carbon black, Vulcan XC-72; Fuel Cell Store) catalyzed air cathode, prepared as previously described (Cheng et al., 2005). A 1000 Ω external load was applied to the external circuit during MFC operation, and anodes were enriched in MFCs for over one month. Enriched anodes were transferred from MFCs into cube MECs and switched to fermentation effluent (C-WW). The MFC pre-acclimation method was compared to anodes that were started directly in MECs, with fermentation effluent as substrate and domestic wastewater inoculum, without anode pre-acclimation in MFCs (C-FW+WW). Domestic wastewater inoculum was added to C-FW+WW reactors in a 1:1 ratio with substrate for the first cycle, with a decreasing inoculum/substrate ratio for the following cycles. Inoculum was omitted once current generation was sustained above 3 mA, which occurred after 3 cycles.

Operation and Measurements

Mini and cube MECs were operated in a 30°C controlled temperature room. The anode and cathode of each reactor were connected to a programmable power supply (Model 3645A; Circuit Specialists Inc., Mesa, AZ, USA) with an applied potential of 0.7 V for mini MECs and 0.9 V for cube MECs (Cusick et al., 2010; Ren et al., 2013). A digital multimeter (Model 2700; Kiethley Instruments Inc., Cleveland, OH, USA) recorded voltage measurements for each reactor across a 10 Ω resistor placed in series between the anode and positive terminal on the power supply.

Voltage measurements were recorded every 10 minutes on a computer. Ohm's law (*U=IR)* was used to calculate current, while current density (*j*; A/m^2) was normalized to the projected cathode area and averaged over the time to reach 90% charge accumulation ($I_{avg\text{-}90}$), as previously described (Ivanov et al., 2013). The total charge recovered over a batch cycle was calculated by integrating the current over the cycle length ($C_T=\sum I \cdot \Delta t$; C). Coulombic efficiency (CE) was based on the total charge measured and change in chemical oxygen demand (COD) over a cycle (Ivanov et al., 2013).

Substrate was replaced when current decreased below 0.02 mA in mini MECs and 0.2 mA in cube MECs. Gas volume and composition in the cube MECs was determined using a gas bag method based on initial nitrogen gas concentrations (Ambler & Logan, 2011). Hydrogen, methane and nitrogen concentrations were determined using a gas chromatograph (Model 310; SRI Instruments, Torrance, CA, USA) with a 6-foot molecular sieve packed 5A column, and argon as a carrier gas. Carbon dioxide was quantified using a GC with a 6-foot porapak Q column, and helium gas carrier. Reactor headspace and gas bags were sampled with an airtight syringe (0.5 mL Gastight syringe, Hamilton Co., Reno, NV, USA). Gas quantity was determined using the known headspace volume (10 mL) and a gas bag method based on initial nitrogen gas concentrations (Ambler & Logan, 2011). Cathodic and overall gas conversion efficiencies for methane and hydrogen combined and hydrogen only were calculated as previously described (Wagner et al., 2009). Between cycles, the headspace in mini MECs was sparged with an 80:20 N_2/CO_2 gas mixture for 2 minutes and cube MEC headspace was sparged for 20 minutes with ultra-high purity N_2 gas. Cube MEC gas bags were sparged by filling with ultra-high purity N_2 gas and vacuuming empty three times in succession.

Protein concentrations were determined using a bicinchoninic acid assay (Pierce BCA; Thermo Scientific, Rockford, IL, USA) with standard test-tube procedure. Bovine serum albumin (BSA) was used as a protein standard, and samples were incubated at room temperature for 2 hours. Absorbance at 562 nm was measured using a UV spectrophotometer (Model DR2700; Hach Co., Loveland, CO, USA) and compared to BSA standards to determine concentration. Chemical oxygen demand (COD) was measured using a standard chromic acid colorimetric method (Hach Co., Loveland, CO, USA).

4.4 Results and Discussion

Effect of Acclimation Method on Treatment Performance

At least 79% of COD was removed in all MECs, with generally greater removal in mini MECs (81-86%) than cube MECs (79-82%) (Figure 4.1). Effluent COD concentrations in the mini MECs were significantly different between acclimation methods (ANOVA, $p<0.01$), with COD removals in pre-acclimated reactors larger than those in non-pre-acclimated ones. The M-WW reactors produced the lowest effluent COD concentration (142 ± 19 mg/L), followed by M-AC (175 ± 25 mg/L), and M-WW+FE (209 ± 47 mg/L). Cube MECs with wastewater acclimated anodes (C-WW) had an average effluent COD concentration of 228 ± 58 mg/L, which was not significantly different (T-test, $p>0.18$) than the average effluent COD measured in the C-FE+WW reactors (262 ± 62 mg/L).

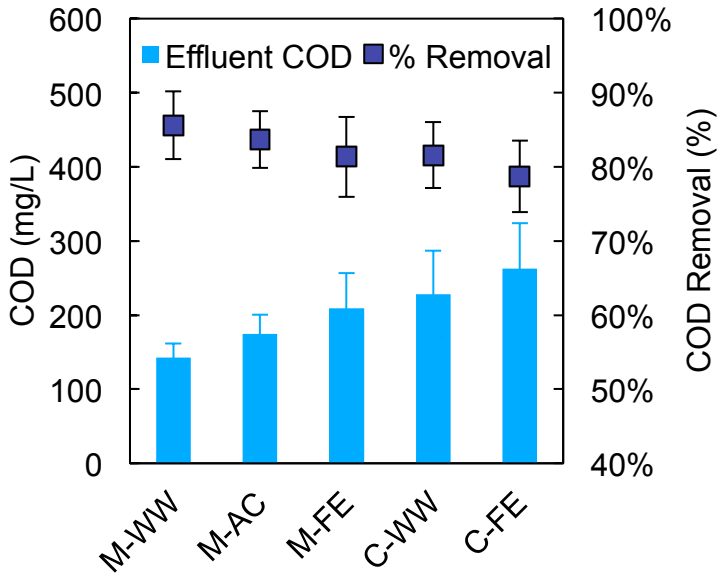

Figure 4.1 Effluent COD concentrations and total COD removal in mini and cube MECs fed fermentation effluent with different acclimation methods.

Treatment performance, as measured by the effluent COD concentration, improved over 22 batch cycles in mini MECs, but wastewater acclimated reactors consistently removed more COD than acetate-acclimated and non-pre-acclimated reactors (Figure 4.2a). Similarly, effluent COD concentration decreased over multiple batch cycles in cube MECs, and C-WW reactors consistently had lower effluent COD concentrations than C-FE+WW reactors over 18 batch cycles (Figure 4.2b). Based on the results in both mini and cube MECs, domestic wastewater acclimated reactors showed slightly improved COD removal compared with acetate-acclimated and non-pre-acclimated reactors, although the difference in effluent COD concentration was greater between reactor types than acclimation methods.

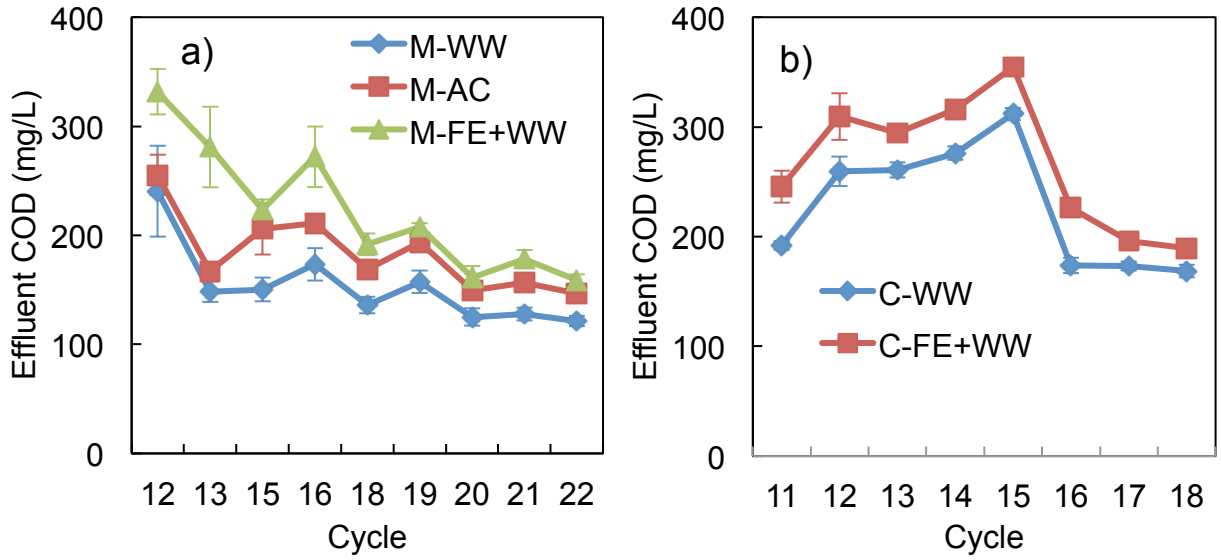

Figure 4.2 Effluent COD concentrations for differently acclimated (a) mini and (b) cube MECs fed fermentation effluent over multiple cycles.

Cube and mini MECs were operated in open circuit mode to measure background COD removal (no current generated). Less than 5% of the influent COD was removed in the mini MEC reactors, and only 11-12% was removed in cube MECs, with no consistent relationship between different acclimation methods (Figure 4.3). Based on these results, nearly all of the organic removal in closed circuit mode could be attributed to anodic oxidation of organic matter, since relatively little COD was removed in open circuit.

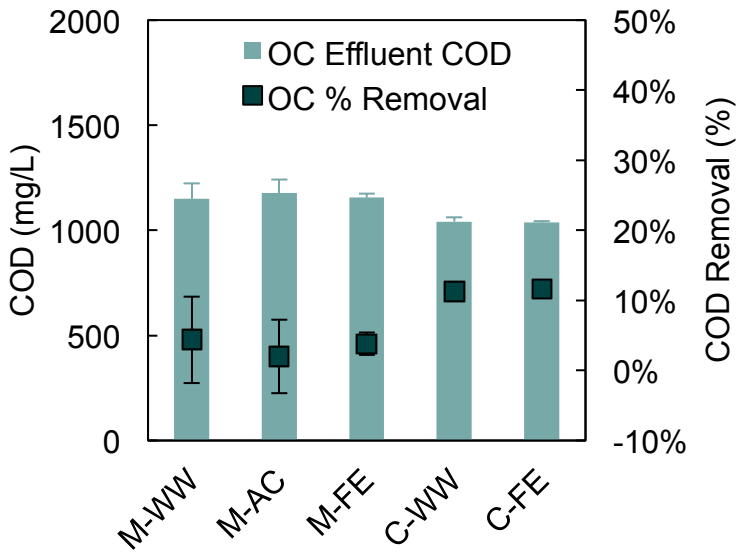

Figure 4.3 Effluent COD concentration and removal in mini and cube MECs during open circuit cycle with no current generation.

Although COD removal varied among mini MECs based on acclimation method, average current densities were very similar regardless of acclimation. M-WW and M-AC reactors both had an $I_{avg\text{-}90}$ of 2.25 A/m^2, while M-AC+WW was slightly lower at 2.20 A/m^2 (Figure 4.4a). Average current densities in cube MECs were higher than those in the mini reactors, likely because the platinum catalyst layer on the cube MEC cathodes reduced the electrode overpotential. The average current in the C-WW reactors was 3.77 ± 0.93 A/m^2, which was not significantly different (T-test, $p>0.69$) than current generated in the C-FE+WW reactors (3.89 ± 0.45 A/m^2). Since COD removals varied, but current generation remained nearly constant among reactors with different acclimation methods, the enhanced COD removals of the M-WW reactors was attributed to direct methanogenesis, and not increased anodic oxidation.

Coulombic efficiencies were not significantly different between mini MEC acclimation conditions (ANOVA, $p>0.09$), with averages of 74-76% (Figure 4.4b). For the cube MECs, CE

was significantly different between the acclimation methods (T-test, $p<0.002$). The C-FE+WW reactors had a CE of 82 ± 4%, which was greater than the CE for the C-WW reactors (76 ± 4%).

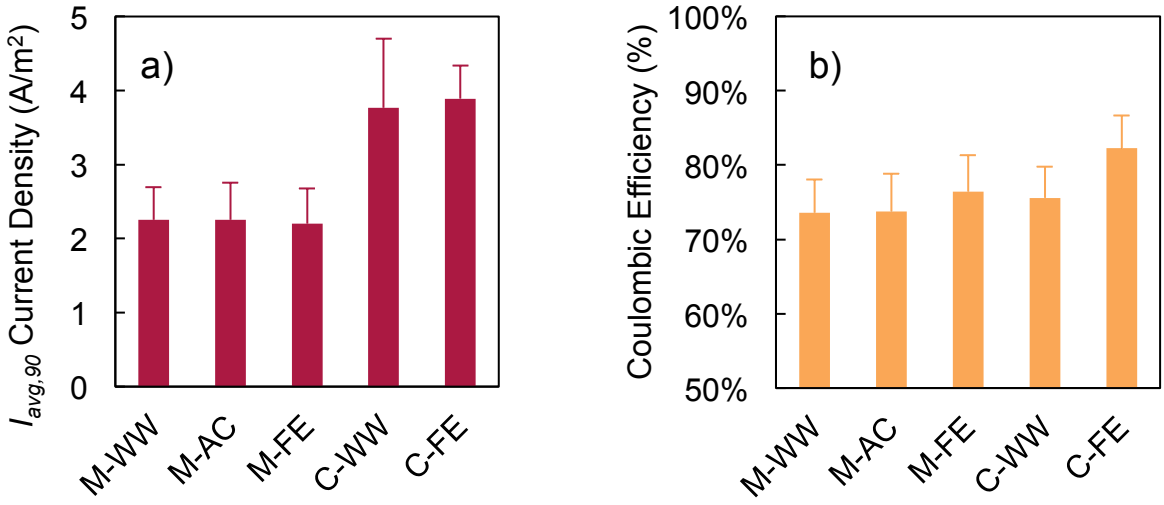

Figure 4.4 (a) Average current density over the time to 90% charge accumulation (I_{avg-90}) and (b) coulombic efficiency of mini and cube reactors with different acclimation methods fed fermentation effluent.

Gas Recovery and Efficiency

Biogas recovered from the cube MECs primarily consisted of hydrogen. Slightly more total gas was recovered in the C-WW reactors (19.1 ± 2.8 mL) than the C-FE+WW reactors (17.3 ± 4.7 mL), but the difference was not significant due to variations in gas recovery among batch cycles and replicates (Figure 4.5a). Gas composition was not significantly different between the acclimation methods. Recovered gas was approximately 30% methane and 60% hydrogen in both the C-WW and C-FE+WW reactors. Cube MECs were switched to open circuit operation at the end of the experiment to measure background gas production. There was relatively little gas measured after open circuit cycles, and it consisted primarily of methane, with slightly more recovered from the C-FE+WW reactors (3.0±0.3 mL CH_4) than the C-WW reactors (2.5±0.2 mL CH_4).

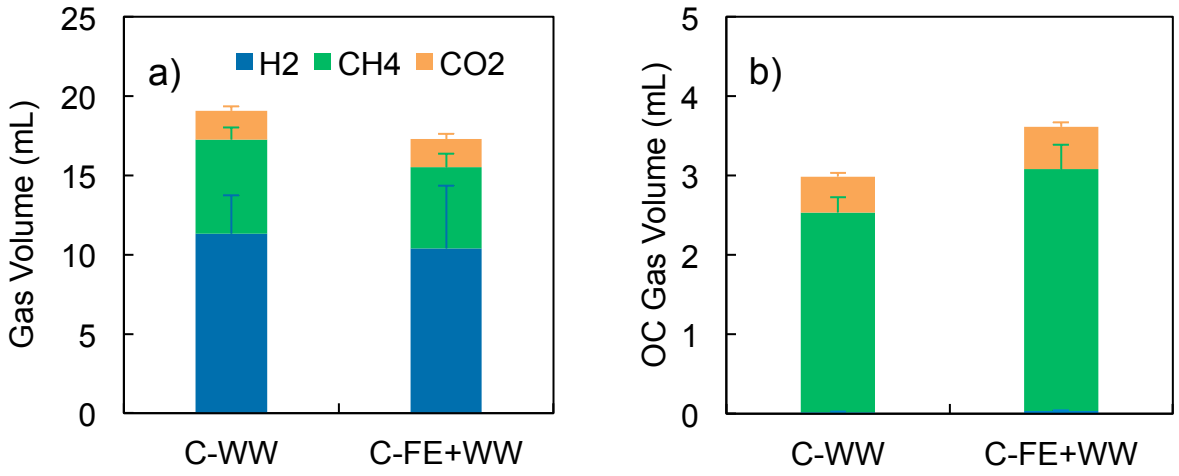

Figure 4.5 (a) Gas recovery in cube MECs fed fermentation effluent with different acclimation procedures during closed circuit cycles and (b) gas recovery after an open circuit cycle.

Cathodic gas recovery ($r_{H2+CH4,\ cat}$), which is the fraction of Coulombs measured as current that were recovered in biogas, was lower for the C-FE+WW reactors (79 ± 12%) than the C-WW reactors (96 ± 10%) (Figure 4.6a). Overall gas recovery ($r_{H2+CH4,\ COD}$), which is the ratio of electrons recovered as gas to those removed as COD, was also lower for the C-FE+WW reactors (65 ± 11%) than the C-WW reactors (72 ± 7%). Thus, the majority of methane generation was likely due to hydrogenotrophic methanogenesis, rather than acetoclastic methanogenesis, because overall and cathodic gas recoveries were below 100%.

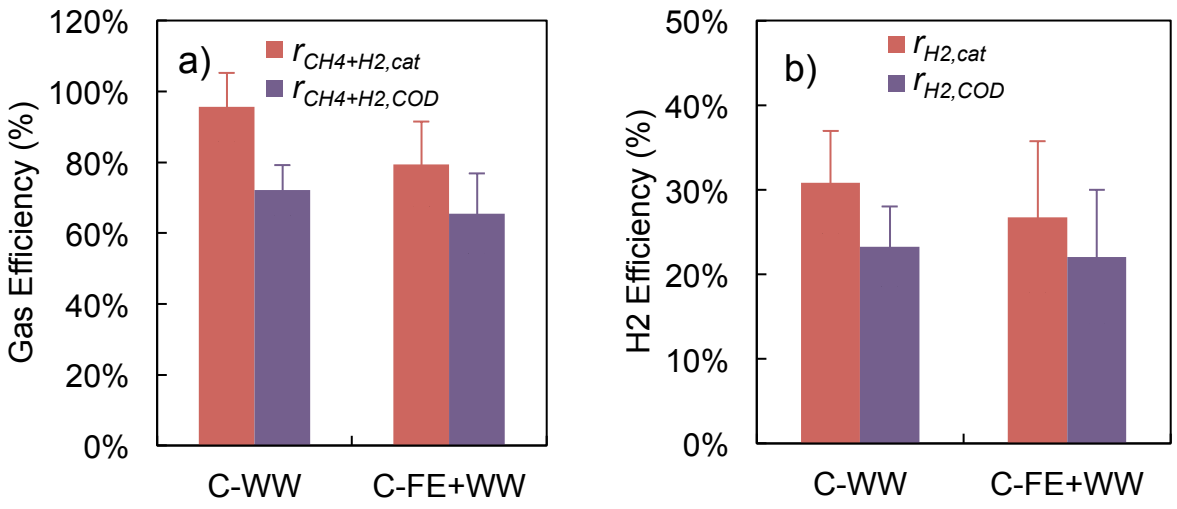

Figure 4.6 Cathodic and overall gas conversion efficiency for cube MECs with different acclimation procedures based on (a) total hydrogen and methane, and (b) total hydrogen gas recovered.

Protein Removal

Measured protein removal was highest in the pre-acclimated mini MECs, with 84 ± 2% of protein removed in the M-WW reactors, followed by the M-AC reactors with 82 ± 2% and the M-FE+WW reactors with 79 ± 2% (Figure 4.7a). Effluent protein concentrations in cube MECs were significantly larger than the mini MECs, with 66% protein removal for both C-WW and C-FE+WW. This indicated that the difference between protein removal in mini and cube reactors was greater than the difference between acclimation methods, which was consistent with the observed difference in COD removal between the mini and cube MECs.

To determine the protein fraction of the total influent and effluent COD, the protein concentrations measured with the BCA test were converted into COD concentrations. The COD of the BSA protein standard was measured as 1.62 g COD/g BSA. Using this conversion factor, the estimated COD of the protein in the cube MEC effluent was higher than the measured

effluent COD (Figure 4.7b). The reasons for this are not clear. Absorbance characteristics can vary between different proteins in the BCA assay (Wiechelman et al., 1988), and since the actual protein composition of the fermentation effluent was not known, the BSA standard may not have been representative of the protein in the fermentation effluent. The color change in the BCA test is generated by the complexation of BCA and Cu^{+}, so other compounds that can reduce Cu^{2+} to Cu^{+} would produce a color change (Wiechelman et al., 1988). Since the fermentation effluent was a complex sample, it is possible that interfering compounds contributed to the measured protein concentrations.

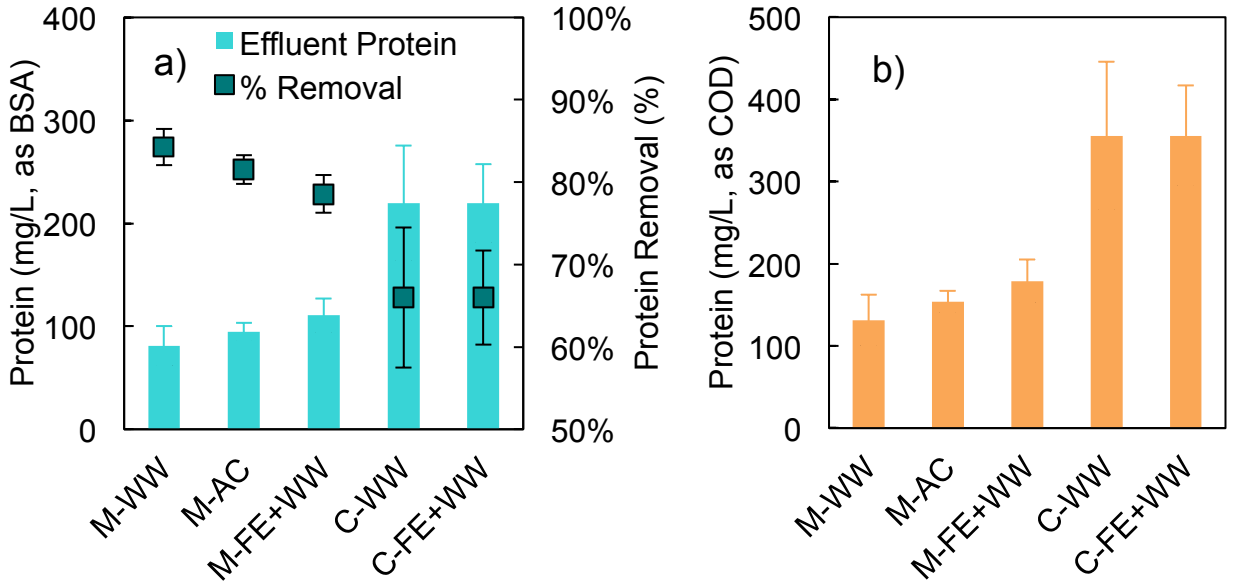

Figure 4.7 (a) Effluent protein and total protein removal in mini and cube MECs measured using the BCA assay with BSA standard. Average influent protein concentration was 560 ± 80 mg/L as BSA. (b) Effluent protein concentrations converted to COD using conversion factor of 1.62 g COD/g BSA.

4.5 Conclusions

Acclimating MEC anodes to acetate or wastewater before tests using fermentation effluent slightly improved COD removal, but acclimation had little effect on current generation. In both mini and cube MECs, acclimating anodes to domestic wastewater prior to treating fermentation effluent (M-WW, C-WW) improved COD removal compared to reactors that were not pre-acclimated (M-WW+FE, C-WW+FE). Average current density was not significantly influenced by acclimation method, and differences in coulombic efficiency were <7%. Differences in protein removals were relatively small between different acclimation methods in mini MECs, with no differences measured between cube MEC acclimation methods. Although acclimation improved the extent of treatment with both mini and cube MECs, the difference in COD treatment, current generation, and protein removal was more significant between mini and cube MECs than different acclimation methods. These results suggest that different acclimation methods have less influence on MEC performance than reactor materials and design. Acclimating reactors to wastewater produced an anodic biofilm that was stable and performed as well as, or better than, acetate acclimated reactors treating fermentation effluent, indicating that expensive acetate amendments may not be necessary for MEC startup if a domestic wastewater source is available.

5. FUTURE WORK

To improve our understanding of MEC processes and continue towards practical implementation in real world environments, additional studies should focus on the following areas:

- Correlate performance between larger bench and pilot reactors with mini and cube MECs to better determine how gas production, current generation, and organic removal changes with scale-up.
- Compare continuous and batch mode operation with actual wastewater samples to better understand the effects of process configurations on large-scale reactor designs.
- Examine organic loading rates with high-strength industrial effluents by dilution of the sample, while maintaining conductivity, to determine if current generation, organic removal and gas production rates could be optimized.
- Evaluate COD concentrations and gas production over time to determine optimal cycle time for the greatest hydrogen production efficiency and lowest input energy requirements.
- Compare treatment performance with various set anode potentials in MECs to determine maximum COD removal with real wastewater samples.

APPENDIX

Supplemental Information

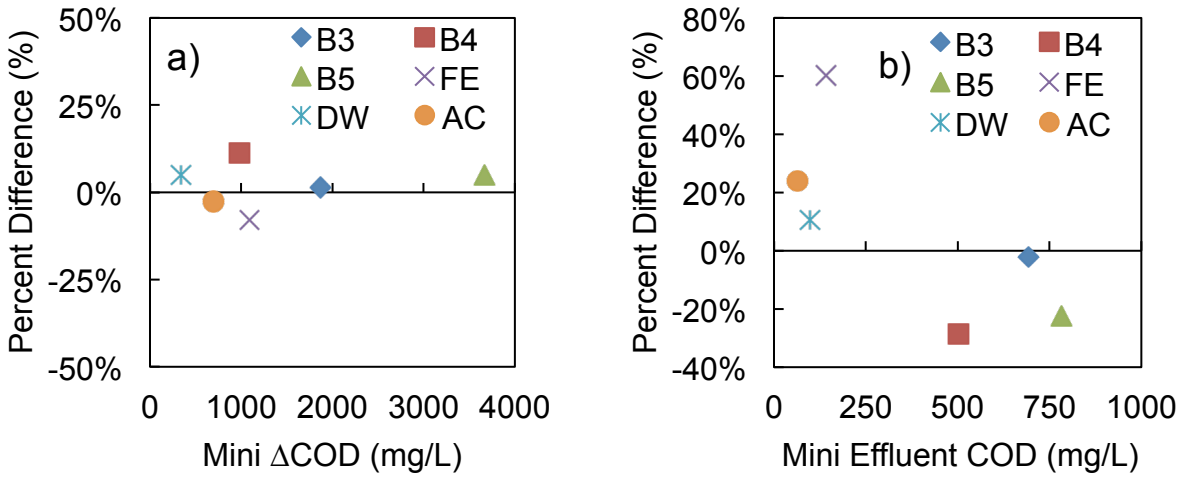

Figure S.1 Residual percent difference between cube and mini MEC measurements of (a) total COD removal and (b) effluent COD.

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