M-WW

Operated with DW and switched to FE

M-AC

Operated with AC and switched to FE

M-FE

Not pre-acclimated, started on FE with DW inoculum

C-WW

Anodes enriched in MFCs fed DW and transferred to MECs

C-FE

Not pre-acclimated, started in MECs with DW as an inoculum

Pre-acclimation improved COD removal

No influence on current

Date: March 15, 2015
Submitted to: *International Journal of Hydrogen Energy*

Anode Acclimation Methods and Their Impact on Microbial Electrolysis Cells Treating Fermentation Effluent

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ABSTRACT

There is a lack of standardized acclimation procedures for evaluating treatability of different wastewaters, and such tests are often conducted using different types of microbial electrolysis cells (MECs). Two different types of MECs (mini or cube) were therefore acclimated using two different substrates (acetate or domestic wastewater) to see the impact of these procedures on the resulting treatment efficiency using the same cellulose fermentation effluent. COD removal was slightly larger using mini MECs (81-86%) than cube MECs (79-82%). Pre-acclimation of mini MECs to domestic wastewater increased COD removal slightly compared to non-acclimated tests with fermentation effluent, but acclimation differences for the cube MECs were not statistically significant. Gas production was not significantly different for cube pre-acclimated MECs compared to those acclimated only to the fermentation effluent. Current densities were higher for the cube reactors than the mini MECs, but they were unaffected by acclimation procedure (pre-acclimation or direct use of fermentation effluent). These results show that mini MECs acclimated to a readily available complex source of organic matter (domestic wastewater) can produce equivalent or slightly superior results for tests with a different complex wastewater (fermentation effluent), and that mini MEC performance is comparable to that of cube MECs.

The similarity of reactor performance allows the use of simple and inexpensive mini MECs that can be acclimated to domestic wastewater and subsequently used to test different types of industrial effluents.

KEYWORDS: Microbial Electrolysis Cells; Fermentation Effluent; Mini Microbial Electrolysis Cells; Acclimation

1. Introduction

Advances in anaerobic biological treatment technology have redefined what can be considered “waste” by demonstrating that useful products can be generated or recovered from a wide range of domestic, industrial and agricultural byproducts [1, 2]. Electricity, hydrogen, methane and various chemicals can be generated through these processes, but hydrogen is especially attractive because it is a valuable product that has a high energy density and it has broad use in different industrial applications [3-5]. Waste products, such as crop biomass, food waste, and industrial wastewaters rich in carbohydrates can be used as renewable energy sources to produce hydrogen gas by dark fermentation [6-11]. However, while one mole of glucose can stoichiometrically be converted to 12 moles of hydrogen, maximum yields of only 2-3 mol H₂/mol glucose are typically observed because other end products, like acetate and butyrate, are also produced along with hydrogen [1, 7, 9, 12]. The effluent from a dark fermentation process is therefore rich in organic acids, ethanol, and other organics that cannot be further fermented to produce hydrogen, which limits conversion efficiencies and energy recovery [8, 11, 13].

To improve overall yields of substrate conversion to hydrogen gas, dark fermentation processes can be integrated with post treatment systems such as microbial electrolysis cells (MECs) [12]. MECs utilize exoelectrogenic microbes that can readily convert organic acids, such

as acetate, into electrical current, making them useful for treating fermentation effluent and recovering additional energy [14-17]. Exoelectrogenic microbes form a biofilm on a conductive anode, which is coupled with a hydrogen-evolving cathode to complete the cell. MECs require a source of electrical power to drive the hydrogen evolution reaction at the cathode, with 0.5-1.0 V of additional potential typically applied to supplement the potential generated by the anode [18-21]. This power can be generated by renewable sources, such as solar and wind, or from salinity gradient energy derived from natural or artificial solutions [22, 23].

A combined treatment process using a small (cube type) MEC with a dark fermentation effluent was shown to increase hydrogen yields to nearly 10 mol H₂/mol glucose from a cellobiose feedstock, compared to 1.65 mol H₂/mol glucose by dark fermentation alone [24]. However, this comparison was made on the basis of only volatile fatty acids and alcohols. Fermentation of cellulosic substrates can also result in a high concentrations of protein in the effluent due to the production of cellulosomes, for example by *Clostridium thermocellum*, that are needed to break down the cellulose into sugars [37, 38]. Proteins can be degraded in MECs, but they have been infrequently studied in these systems [36, 38]. Since proteins can be used as a substrate in MECs, their concentrations will also be important when examining reactor performance on the basis of COD removal.

MECs used in tests with complex wastewaters have been acclimated using different approaches, but the impact of these different methods has not been well studied for complex wastewaters such as fermentation effluents. When a single substrate such as acetate is used in an MEC, it has been shown that using effluent from a reactor pre-acclimated to that substrate improves performance [39, 40]. However, in some studies with complex wastewaters, for example, fermentation effluent of glycerol and molasses wastewaters, the reactors were

acclimated using only acetate prior to tests on these wastewaters that contained a rich mixture of alcohols and volatile fatty acids [10, 25]. The impact of pre-acclimation to the fermentation effluent or another complex wastewater was not examined. It has been shown by others that pre-acclimation of MECs to a complex source of organic matter and high concentrations of bacteria (domestic wastewater) improves performance of MECs treating an industrial wastewater compared to reactors acclimated only to the industrial wastewater [41, 42]. The microbial diversity of anode communities is known to increase for complex organic matter sources compared to single substrates. Exoelectrogenic microbes that produce electricity in MECs can only use a relatively limited number of different substrates [43, 44], and therefore microbial communities that develop in reactors fed a single substrate, such as acetate, are primarily dominated by various *Geobacter* species [45-47]. However, communities that develop in reactors fed a complex source of organic matter, such as domestic wastewater, are much more rich and diverse [14, 27, 28, 48]. Therefore, it is not known to what extent the pre-acclimation process can affect the performance of MECs treating complex wastewaters such as fermentation effluents.

Various types of MECs have been used to convert residual organic matter fermentation effluent into hydrogen [10, 13, 24-26], but there have been no comparisons of treatability using these different types of reactors. Most tests on fermentation effluents have been done using small, cube-shaped reactors with 25-32 mL per single-chamber, fed-batch test [13, 24, 32-34], although some have also been done using two-chamber MECs [10, 35] or continuous flow conditions [36] that can use larger volumes of 350 mL per batch [36] or 137 mL for a set hydraulic retention time [36] which is typically one day or less. Inexpensive (~\$1-2 each) mini MEC reactors can be easily manufactured from readily available materials [49]. They also have a

very small liquid volume (5 mL) and can be operated with a large number of reactors in parallel, making them useful for studying a wide variety of conditions or substrates [41, 42]. Cube reactors are relatively expensive to make (\$100 each or more) but they use somewhat larger volumes of liquid (which typically must be transported from distant sites to the laboratory). For example, a two-month long test using cube reactors in duplicate (assuming new solution every two days, and 32 mL for each reactor) would require ~2 liters of a sample, compared to less than half a liter for mini-MECs run in triplicate. To reduce liquid sample collection and shipments, tests with cube MECs are often made with a single reactor (sometimes duplicates) but not in triplicate. Mini MECs could be used as a less expensive platform for treatability testing, and reduce volumes of samples that need to be shipped, but the two types of reactors have not been previously compared using an industrial wastewater or fermentation effluent.

The goals of this study were to examine the impact of pre-acclimation procedures (using different inocula), and to compare two different reactor configurations relative to treatment efficiencies using a cellulose fermentation effluent. We compared commonly used MEC pre-acclimation procedures for complex effluents, based on first using acetate or domestic wastewater [18, 42], with acclimation of MECs only to fermentation effluent. The two reactor types examined here were inexpensive small-volume mini MECs that utilize commonly available parts and materials, and more standardized cube-type reactors. Performance of these MECs was evaluated in terms of COD and protein removal, along with gas recovery and coulombic efficiencies.

2. Materials and Methods

2.1 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, USA [11]. Effluent samples were stored on ice and shipped overnight to University Park, PA, USA. 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 in University Park, PA, USA (~ 460 mg/L COD, 0.9 mS/cm). Fresh domestic wastewater samples were collected at least every 2 weeks, and stored at 4°C prior to use. The acetate medium used in MEC acclimation consisted of 1 g/L of sodium acetate dissolved in 50 mM PBS (PBS; 2.45 g/L NaH_2PO_4 , 4.58 g/L Na_2HPO_4) with additional nutrients (0.31 g/L NH_4Cl , 0.13 g/L KCl, trace vitamins and minerals) added (4.0 mS/cm) [55].

2.2 Reactor Construction

Mini MECs were constructed as previously described [49] using 5 mL glass serum bottles (Wheaton, Millville, NJ, USA) as the reactor body, graphite block anodes ($1.5 \times 1 \times 0.32$ cm, Grade GM-10; GraphiteStore.com, Inc., Buffalo Grove, IL, USA) and stainless steel mesh cathodes (1.5×1 cm, Type 304, 50 $\times$ 50 mesh size; McMaster-Carr, Elmhurst, IL, USA). 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.

Cube MEC were constructed from 4-cm polycarbonate cubes, and contained a 3-cm diameter cylindrical anode chamber (32 mL liquid volume), with a 1.6-cm diameter by 7-cm tall glass tube glued to the top of the reactor to provide headspace [18]. The anodes were carbon fiber brushes made using twisted titanium wire current collectors (2.5-cm diameter by 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 [56]. Cathodes were constructed from stainless steel mesh (Type 304, 50 x 50 mesh size; McMaster-Carr, Elmhurst, IL, USA) with a Pt catalyst layer [0.5 mg/cm² platinum, (10% w/w); carbon black, Vulcan XC-72, Fuel Cell Store College Station, TX, USA; and a Nafion binder (5% w/w), 33.33 µL/cm²; Sigma Aldrich, St. Louis, MO, USA] painted onto the liquid facing side of the cathode [57]. A gas collection bag was connected to the headspace of the cube MECs with rubber tubing and needles used to connect the bag to the headspace. Cube MECs were run in duplicate.

2.3 Anode Pre-acclimation

Mini MECs were operated with either domestic wastewater (M-WW) or acetate media (M-AC) as substrate for ~2 months to enrich the anodic biofilm prior to tests with fermentation effluent. Effluent from an MFC reactor was used to inoculate the M-AC reactors to provide an adequate microbial seed, with effluent added in a 1:1 ratio with the acetate medium and was omitted once current production reached at least 0.5 mA. Inoculum addition was not necessary for the M-WW reactors. After the acclimation period in which the reactors produced stable, repeatable current

over multiple cycles, the substrate fed to the M-WW and M-AC reactors was switched from domestic wastewater or acetate to the fermentation effluent sample. This anodic biofilm pre-acclimation procedure was compared to mini MECs that were only fed fermentation effluent with domestic wastewater inoculum, and therefore they did not have a pre-developed anodic biofilm prior to acclimation with the fermentation effluent (M-FE). Domestic wastewater inoculum was added to the M-FE reactors in a 1:1 ratio of fermentation effluent to wastewater, with the wastewater omitted once current production reached at least 0.5 mA.

Carbon fiber brush anodes used in cube MECs were acclimated using different procedures. In one set of tests, brush anodes were first pre-acclimated in MFCs using only domestic wastewater as the substrate and inoculum [18]. MFCs used for these tests were 4-cm polycarbonate chambers with the same dimensions as the cube MECs, containing air cathodes made with a Pt catalyst (0.5 mg/cm^2 platinum, 10% w/w on carbon black, Vulcan XC-72; Fuel Cell Store) prepared as previously described [58]. A 1000Ω external load was used as the external resistor during MFC operation, with anodes enriched in MFCs for over one month. Enriched anodes were transferred from MFCs into cube MECs and immediately switched to fermentation effluent (C-WW). The MFC pre-acclimation method was compared to anodes that were started directly in MECs, with a mixture of fermentation effluent and domestic wastewater, without any other anode acclimation (C-FE). Domestic wastewater inoculum was added to C-FE reactors in a 1:1 ratio with substrate for the first cycle, with a decreasing inoculum/substrate ratio for the following cycles. Wastewater was omitted once current generation was sustained above 3 mA, which occurred after 3 cycles.

2.4 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 128 Specialists, Inc.) with an applied potential of 0.7 V for mini MECs and 0.9 V for cube MECs [42, 59]. A digital multimeter (Kiethley Instruments, model 2700) 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/m²) was normalized to the projected cathode area and averaged over the time to reach 90% charge accumulation ($I_{\text{avg-90}}$), as previously described [41]. 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 [41].

Substrate was replaced when current decreased below 0.02 mA in mini MECs and 0.2 mA in cube MECs [42, 60]. The current density thresholds for substrate replacement were based on previous studies and differ between reactors because of electrode sizes. Gas volume and composition in the cube MECs was determined using a gasbag method based on initial nitrogen gas concentrations [61]. Hydrogen, methane, and nitrogen gas 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). Between cycles, the headspace in mini MECs was sparged with 80:20 N₂/CO₂ anaerobic

gas mixture for 2 minutes and cube MEC headspace was sparged with ultra-high purity N₂ gas for 20 minutes. Cube MEC gas bags were sparged by filling with ultra-high purity N₂ 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 (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 (High range COD vials, Hach Co.).

3. Results and Discussion

3.1 Effect of Reactor Type and Acclimation Method on Treatment Performance

At least 79% of the COD was removed in all MECs, with slight differences between reactor types and pre-acclimation methods. Pre-acclimated mini MECs (M-WW and M-AC) produced slightly lower effluent COD (ANOVA, $p < 0.01$) than reactors that were not pre-acclimated (M-FE) (Figure 1). The M-WW reactors produced the lowest effluent COD concentration (142 ± 19 mg/L), followed by M-AC (175 ± 25 mg/L), and M-FE (209 ± 47 mg/L). For the cube MECs, wastewater acclimated anodes (C-WW) had an average effluent COD concentration of 228 ± 58 mg/L (82% removal), which was lower but not significantly different (T-test, $p = 0.18$) than the average effluent COD measured in the C-FE reactors (262 ± 62 mg/L, 79% removal). COD removals obtained using mini MECs (81-86%) were about 2-4% larger on average than cube MECs (79-82%) for similar acclimation conditions (T-test, $p < 0.05$). These COD removals observed in this study compare favorably to the 81-91% removal efficiency reported by Nam et

al. [24, 36] using a continuous flow reactor and are an improvement over the 65% COD removal efficiency reported by Lalaurette et al. using cube MECs with cellulosic dark fermentation effluent as the substrate [24].

Figure 1. Effluent COD concentrations and total COD removal in mini (M) and cube (C) MECs fed fermentation effluent with different acclimation methods (pre-acclimation to wastewater, WW; pre-acclimation to acetate, AC; or no pre-acclimation using fermentation effluent directly, FE). Influent concentration of diluted fermentation effluent was 1230 ± 70 mg/L of COD.

Treatment performance, as measured by the effluent COD concentration, improved over time (22 batch cycles, over ~3 months) for the mini MECs. Wastewater-acclimated reactors consistently produced lower COD effluent concentration than acetate-acclimated and non-pre-acclimated reactors throughout the experiment, although effluent COD concentration decreased in all reactors over time and the difference compared to the overall COD removal was relatively small (Figure 2a). Effluent COD concentrations were more variable over time with the cube MECs (18 batch cycles, over ~2 months), increasing from cycle 11 to 15 in both C-WW and C-FE reactors before decreasing in cycles 16-18 (Figure 2b). C-WW reactors had generally lower effluent COD concentrations than C-FE reactors over the course of the experiment, which was

consistent with the COD removal results observed in mini MECs. Domestic wastewater acclimated reactors showed slightly improved COD effluent quality compared with acetate-acclimated and non-pre-acclimated reactors in both mini and cube MEC tests (T-test, $p < 0.01$), although the difference in effluent COD concentration was greater between reactor types than acclimation methods. Pre-acclimated reactors (M-WW, M-AC, C-WW) also produced more rapid treatability results since non-pre-acclimated reactors (M-FE, C-FE) required inoculum for the first 3 cycles.

Figure 2. Effluent COD concentrations for differently acclimated (a) mini (M) and (b) cube (C) 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 the different acclimation methods (Figure 3). Based on these results, nearly all of the organic removal in closed circuit mode (with hydrogen production) could be attributed to anodic oxidation of organic matter, since relatively little COD was removed under open circuit conditions.

Figure 3. Effluent COD concentration and removal in mini and cube MECs during open circuit (OC) cycle with no current generation.

3.2 Current Generation and Coulombic Efficiency

Although COD removal varied among mini MECs based on acclimation method, average current densities in the mini MECs were very similar regardless of acclimation procedures. M-WW and M-AC reactors both had average current densities of $I_{\text{avg-90}} = 2.25 \text{ A/m}^2$ (68 A/m^3), while M-FE was slightly lower at 2.20 A/m^2 (66 A/m^3) (Figure 4a). Average current densities in cube MECs were higher than those in the mini reactors, likely because platinum was used as a cathode catalyst in the cube MECs to reduce the electrode overpotential. The average current in the C-WW reactors was $3.77 \pm 0.93 \text{ A/m}^2$ ($82 \pm 20 \text{ A/m}^3$), which was not significantly different (T-test, $p=0.69$) than current generated in the C-FE reactors ($3.89 \pm 0.45 \text{ A/m}^2$, $85 \pm 10 \text{ A/m}^3$). Since COD removals varied, but current generation remained nearly constant among the mini MEC reactors with different acclimation methods, the enhanced COD removals of the M-WW reactors was attributed to methanogenesis, and not substrate removal by increased anodic oxidation of organic matter.

In the mini MECs, coulombic efficiencies (CE) were not significantly different based on acclimation conditions (ANOVA, $p=0.09$), with average CEs of 74-76% (Figure 4b). CE was significantly higher (T-test, $p<0.002$) in the C-FE reactors ($82 \pm 4\%$) than the C-WW reactors ($76 \pm 4\%$). Although this may seem counterintuitive since both I_{avg-90} and effluent COD were not significantly different between the C-WW and C-FE reactors, the CE does not reflect the greater variation in those parameters since it is the ratio of removed COD that is measured as current and varies independently.

Figure 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.

3.3 Gas Recovery and Conversion Efficiency in Cube MECs

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 reactors (17.3 ± 4.7 mL), but the difference was not significant due to variations in gas recovery among batch cycles and replicates (Figure 5a). Gas composition was also not significantly different between the acclimation methods. Recovered gas was approximately 30% methane and 60% hydrogen in both the C-WW and C-FE reactors.

The hydrogen yields were 352 ± 72 mL H_2 /g COD for the C-WW reactors and 334 ± 120 mL H_2 /g COD for the C-FE reactors (Figure 5b), which were lower than those previously reported for cellulose fermentation effluent [24, 36]. These lower yields were a result of higher rates of methanogenesis, with methane yields of 185 ± 21 mL CH_4 /g COD in the C-WW reactors and 164 ± 21 mL CH_4 /g COD in the C-FE reactors. Hydrogenotrophic methanogenesis is difficult to suppress in single chamber systems without the use of chemical inhibitors and hydrogen transport from the dissolved to gas phase can be slower than methanogenic reactions [52, 53, 62]. It is possible that the acclimation methods used in this study, which utilized DWW as inoculum and substrate in C-WW reactors and inoculum in C-FE reactors, were less effective at selecting against methanogenic microbes than acetate acclimation, which was used in the previous studies. Various techniques can be used to limit or inhibit methane production, such as adding inhibitors or using two-chamber systems [53, 54], but these were not examined here as the primary focus was on treatment efficiency based on COD and protein removal.

Cube MECs were switched to open circuit operation at the end of the experiment to measure background gas production in the absence of current generation. There was relatively little gas measured after open circuit cycles, and it consisted primarily of methane, with slightly more recovered from the C-FE reactors (3.0 ± 0.3 mL CH_4) than the C-WW reactors (2.5 ± 0.2 mL CH_4) (Figure 5c). The presence of methane during open circuit operation indicates that some acetoclastic methanogenesis could have occurred during closed circuit cycles, but the extent to which it contributed to methane recovered during closed circuit cycles, when hydrogen was produced, is not known. It is generally thought that anode respiring microbes outcompete acetoclastic methanogens while current is generated [63], but there was no conclusive way to

differentiate between methane generated by acetoclastic or hydrogenotrophic microorganisms in this study.

Figure 5. (a) Gas recovery in cube MECs fed fermentation effluent with different acclimation procedures during closed circuit (CC) and open circuit (OC) cycles and (b) average hydrogen and methane yields. Average cycle time for cube MECs was 3.0 ± 0.4 days.

Cathodic gas recovery ($r_{H_2+CH_4, cat}$), which is the fraction of Coulombs measured as current that were recovered in biogas, was significantly (T-test, $p < 0.01$) lower for the C-FE reactors ($79 \pm 12\%$) than the C-WW reactors ($96 \pm 10\%$) (Figure 6a). Overall recovery ($r_{H_2+CH_4, COD}$), which is the ratio of electrons recovered as gas to those removed as COD, was also lower for the C-FE

330 reactors ($65 \pm 11\%$) than the C-WW reactors ($72 \pm 7\%$). Combined recoveries were calculated
331 assuming that recovered methane was produced via hydrogenotrophic methanogenesis, which
332 requires 4 moles of hydrogen per mol of methane. Cathodic gas recovery was less than 100% in
333 both C-WW and C-FE reactors, indicating that most of the methane generated in closed circuit
334 cycles was likely generated by hydrogenotrophic methanogens. Methane from acetoclastic
335 microbes would have contributed to gas recovery without generating current. The lower
336 $r_{\text{CH}_4+\text{H}_2,\text{cat}}$ in the C-FE reactors could be the result of side reactions, such as hydrogen cycling, but
337 it is not clear what specifically caused it to be lower than C-WW reactors. Cathodic and overall
338 gas recoveries based only on hydrogen ($r_{\text{H}_2,\text{cat}}$, $r_{\text{H}_2,\text{COD}}$) were not significantly different (Figure
339 6b).

Figure 6. (a) Cathodic ($r_{\text{H}_2+\text{CH}_4, \text{cat}}$) and overall ($r_{\text{H}_2+\text{CH}_4, \text{COD}}$) recovery of hydrogen and methane and (b) cathodic ($r_{\text{H}_2, \text{cat}}$) and overall ($r_{\text{H}_2, \text{COD}}$) recovery of hydrogen based on COD and current for cube MECs with different acclimation procedures.

3.4 Protein Removal

Protein removal was examined and compared with COD removal to determine if differences in organic removal between the acclimation methods were the result of changes in protein utilization. Measured protein removal was highest in the pre-acclimated mini MECs, with $84 \pm 2\%$ of protein removed in the M-WW reactors, followed by the M-AC reactors with $82 \pm 2\%$ and the M-FE reactors with $79 \pm 2\%$ (Figure 7a). This is consistent with the COD results and

suggests that at least some of the increased COD removal observed for the pre-acclimated reactors (M-WW, M-AC) could be attributed to increased protein utilization in the mini MECs. This difference was not observed in the cube MECs, with 66% protein removal in both the C-WW and C-FE reactors. Although organic removal was also higher in the cube MECs, the difference between the mini and cube MEC protein removal rates is greater than would be expected based on the COD removal results.

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 7b). The reasons for this are not clear. Absorbance characteristics can vary between different proteins in the BCA assay [64], 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 [64]. Since the fermentation effluent is a complex sample, it is possible that interfering compounds contributed to the measured protein concentrations.

Figure 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. Conclusions

Mini MEC and cube MECs were generally similarly effective for evaluating COD removal and treatability of the fermentation effluent, although COD removals were sometimes slightly larger in mini MECs than cube MECs. Acclimating mini MECs to acetate or wastewater prior to tests using fermentation effluent produced only a small improvement in COD removal (3-5%), while pre-acclimation using domestic wastewater in cube MECs did not have an impact on COD

treatment. The different acclimation procedures also had no appreciable impact on current generation, and differences in coulombic efficiency were small ($<7\%$). Differences in protein removals were also relatively small between acclimation methods in mini MECs (3-5%), with no differences measured between cube MEC acclimation methods.

These results show that acclimating mini MECs to domestic wastewater (i.e. using only domestic wastewater as the inoculum) provided comparable results to tests using fermentation effluent acclimation, based on results of COD removal of fermentation effluent. Pre-acclimation to readily available domestic wastewater is preferred, compared to a specific industrial wastewater, because it allows for more rapid treatability results from the time an industrial wastewater is received to when tests can be conducted using that wastewater. Thus, there is no lag in time for beginning treatability studies with different wastewaters. Domestic wastewater acclimated MECs performed at least as well as acetate acclimated reactors, indicating that acetate addition may not be necessary when developing anodic biofilms for treating wastewaters such as the fermentation effluent tested here. It is often difficult and expensive to ship large volumes of wastewater to a laboratory for treatability testing. The use of mini MECs allows treatability tests to be conducted using small volumes of wastewater, with good replication (triplicate samples were used), and the reactors are relative inexpensive to manufacture. The current densities in mini MECs were slightly lower than those in cube reactors, but COD removals are similar or slightly better than those obtained with cube MECs. It is often difficult to evaluate results in the literature on treatability when different types of MECs are used. We conclude that the simplicity and low cost of the mini MECs could make them a standard for all bioelectrochemical treatment tests, and provide a way to benchmark treatability of different wastewaters based on COD removal and coulombic efficiency.

Acknowledgments

The authors would like to thank Pin-Ching Maness and Lauren Magnusson at the National Renewable Energy Lab (NREL) for providing fermentation effluent samples. This work was supported by Department of Energy Cooperative Agreement DE-EE0005750 and award KUS-11-003-13 from the King Abdullah University of Science and Technology (KAUST).

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