

Optimization of a novel flat-plate microbial electrolysis cell (MEC) for swine wastes to
hydrogen

by

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Abstract

As economic volatility and geopolitical conflict marks the 2020s, vulnerabilities in the global supply chain have highlighted the need for diversification in the sourcing of essential resources. These risks have heightened the need for sustainable resource recovery technologies. Hydrogen is an alternative energy source by itself, while also emerging as a key element in petroleum refining, metal treatment, chemical synthesis, fertilizer production, food processing, and as a fuel additive for biomanufactured aviation fuel. This study evaluated the development and performance of a novel flat-plate microbial electrolysis cell (MEC) designed to produce high-purity hydrogen gas from wastes, specifically swine wastewater, which is generated in large quantities by livestock operations. Initially, acetate was used as a substrate, followed by swine wastewater with a Chemical Oxygen Demand (COD) range of 5,000 to 30,000 mg/L. The MEC featured a shared anode chamber with two carbon fiber anodes (total projected area of 300 cm²) and two flanking cathode chambers with stainless steel mesh cathodes. The anode potential was maintained at -0.3 V versus an Ag/AgCl reference electrode, with continuous monitoring of current density, applied potential, and regular sampling of gas production, pH, COD, and volatile fatty acid (VFA) concentrations.

During the acetate trials, the MEC was operated for seven consecutive batch cycles before transitioning to semi-continuous feed conditions. In the batch phase, it achieved a maximum volumetric hydrogen production rate of 0.99 L/L_{cathode}/d (1.02 mol H₂/mol COD), a peak current density of 8.99 A/m² at an applied voltage of 1.32 V, and a maximum coulombic recovery of 78%. Under continuous operation, these values improved significantly, with a hydrogen production rate of 7.29 L/L_{cathode}/d (2.56 mol H₂/mol COD), a current density of 7.31 A/m² at 1.18 V, and a coulombic recovery of 111% (coulombic efficiency of 114%). After continuous acetate

experiments, the system was switched to swine wastewater for six batch cycles with the incorporation of anaerobic microbial consortia pre-enriched with swine wastewater to maintain an overall solids concentration of ~1% total solids (TS) in the anode suspension. Despite the transition to a more complex substrate and a COD shock of 30,000 mg/L on the fourth batch, the MEC rapidly acclimated and achieved a maximum volumetric hydrogen production rate of 3.56 L/L_{cathode}/d (0.83 mol H₂/mol COD), a peak current density of 10.32 A/m² at an applied voltage of 1.35 V, and a coulombic efficiency of 86%. Throughout each phase of operation, high purity hydrogen was maintained, with slight decreases during the swine wastewater batch. The volume/volume headspace hydrogen concentrations were 97±6% for batch acetate, 100±2% for continuous operation, and 95±2% for the swine wastewater phase.

The current densities reported with swine wastewater in the novel flat-plate MEC are among the highest reported values in published literature and it further demonstrated resilience to variations in influent organic concentrations, yielding current densities substantially higher than those of existing wastewater studies. Hydrogen production and current densities demonstrated by the swine wastewater operation show considerable promise for further exploration in real-world applications while lowering the applied voltage to ~0.5 – 0.8V, for energy positive and economically viable operation at scale.

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Chapter 1 - Introduction

1.1 Literature Review

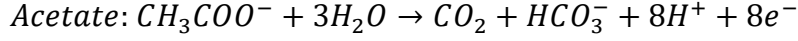
As economic volatility and geopolitical conflict marks the 2020s, vulnerabilities in the global supply chain have highlighted the need for the diversification in the sourcing of essential resources. These risks have heightened the need for sustainable resource recovery technologies. Hydrogen, a key element in petroleum refining, metal treatment, chemical synthesis, fertilizer production, food processing, and as a fuel additive for biomanufactured aviation fuel, has emerged as an important player in the global efforts for economic and environmental resiliency [1], [2]. Another reason for this interest is the high energy density of hydrogen gas. In comparison to other gaseous fuels, hydrogen gas has one of the highest energy densities at 120 MJ/kg versus 44 MJ/kg for gasoline and 50 MJ/kg for methane. As a reflection of this importance, in addition to an already 90 Mt per year demand, the International Energy Agency estimates that demand for H₂ may grow as high as 105 Mt per year by 2030. For net zero emissions goals, an even greater 200 Mt would be required [3]. Currently, as much as 95% of hydrogen gas is produced through steam-methane reforming, a process that utilizes high energy input in the presence of a catalyst at temperatures of 1,300-1800°F and pressures of 3-25 bar to split the hydrogen-carbon bonds in methane to produce hydrogen gas, carbon monoxide, and carbon dioxide [4]. Steam-methane reformed hydrogen, which is largely driven by the combustion of carbonaceous fuel stocks, contributes approximately 900 Mt of carbon dioxide emissions per year and is greatly affected by the price of combustible fuel [3].

Alternatively, hydrogen gas can also be produced by thermochemical, photolytic, or biological processes that use other pathways to split biomass or water into hydrogen gas and byproducts. Electrolysis is a growing process that runs an electrical current through water, splitting it into hydrogen gas and hydroxide ions [4]. Electrolytic hydrogen has an especially high proposition to

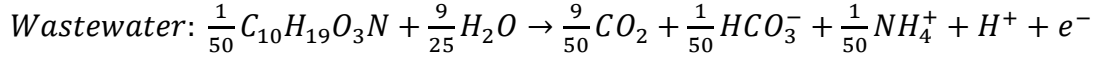
be incorporated into the hydrogen portfolio due to its compatibility with renewable energy sources. In mid-2021, the global electrolysis-derived hydrogen capacity was 300 MW with an additional 54 MW in projects scheduled to be completed by 2030. This increase in production equates to approximately 8 Mt of additional electrolytic hydrogen capacity [3]. To accomplish these goals, there must be continued efforts to develop platforms that achieve scalable sustainable hydrogen production.

1.1.1 Microbial Electrolysis Cells

One of these developing hydrogen production technologies is microbial electrolysis cells (MECs). This platform harnesses the natural processes found in anode-respiring bacteria (ARB) to produce the electrical current required for electrolysis. ARB such as *Geobacter sulfurreducens*, *Geothickilbacter*, and *Thermincola ferriacetica* form biofilms on conductive surfaces and oxidize organic feedstocks to produce electrons. These electrons are then transferred to the surface through extracellular transport (EET) including direct conduction, nanowire transport and redox shuttling, creating a current that can be harnessed for electrolysis or synthesis of other valuable products [5]. Unlike many platforms for hydrogen synthesis, MECs typically only require 0.6-1 kWh/m³-H₂ versus typical energy requirements of 4.5-50.6 kWh/m³-H₂ [6]. One characteristic that lends to the versatility of these platforms is compatibility with a large spectrum of substrates. Acetate has thus far been the preferred substrate for most anode-respiring bacteria due to its simplicity and high bioavailability [7], [8], [9], [10], [11], [12], [13], [14]. While acetate and simple organic substrates have been extensively studied, to be successful at scale, more study needs to be done on complex substrates such as domestic wastewater and high-strength feedstocks that are otherwise treated as waste, losing out on potential opportunities for cost savings in hydrogen production. The oxidation half reactions of acetate and domestic wastewater are given below.



$$E_{anode}^0 = -0.29 \text{ V}$$



$$E_{anode}^0 = \text{varies based on composition}$$

In more complex feedstocks like wastewaters, it is difficult to maintain a culture of strictly ARB, resulting in other competing reactions from suspended organisms. Competing organisms, especially methanogens, utilize electrons that could otherwise be captured as current and can lead to losses in the efficiency of the MEC. Conversely, MECs with complex feedstocks can also take advantage of syntrophic microbes to make the substrate more bioavailable to the ARB. Homo-acetogens including *Acetobacterium*, *Clostridium*, *Alkaliphilium*, *Sporomusa*, and *Eubacterium* can convert fermentation products that are difficult for ARBs to consume into acetate, increasing the bioavailability of the substrate [5]. This process of increasing the bioavailability of complex wastewater products is shown in Figure 1. Wastewater is first hydrolyzed into organic and inorganic macromolecules, then broken down into monomers, which can then be fermented into organic acids and converted to acetate. Methanogenesis is also a possible outcome of this process but should be minimized to ensure electron conversion to the intended cathodic product.

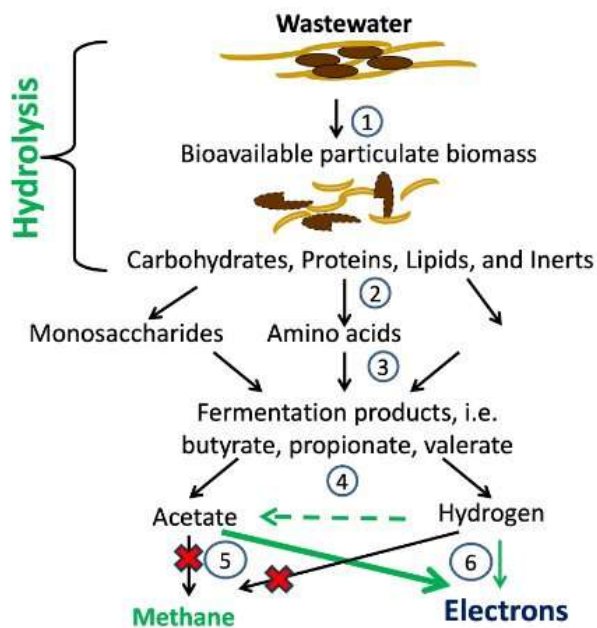
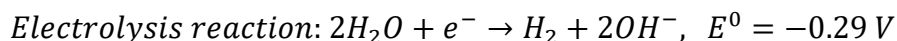


Figure 1: Wastewater fermentation to acetate (Credit: Dr. Parameswaran)

While cathodic half reactions are driven by the current produced at the anode, the chemistry of the hydrogen evolution reaction (HER) is largely independent of the chemistry of the anode half reaction. In HER, current produced is applied directly to the catholyte and splits the water molecules present into hydrogen gas and hydroxide ions as shown in the reaction below.



While a promising area of low-carbon hydrogen production, MEC technology still requires further development to be applied at scale. Pilot and bench-scale systems alike have continued to face many of the same challenges in the areas of overpotential losses and inefficiencies in anode, cathode, and membrane technology [5].

1.1.2 Overpotentials

Overpotentials are the losses that occur due to inefficiencies in MEC design that increase the required energy input for operation. They are typically broken down into anodic, cathodic, ohmic, and concentration overpotentials. Anodic overpotentials (ΔE_{an}) include energy to transfer the

electron from the electron carrier to the anode, the potential difference required to drive EET from the cell to the anode, and the energy used by the ARB for cell growth. This is typically a small component of the potential losses (less than ~ 0.2 V for a healthy biofilm) and is not easily manipulated. Healthy and diverse microbial communities are ideal for ensuring sufficient pathways for EET allowing for more opportunities, and subsequently fewer losses, in transferring electrons to the anode. Cathodic over-potentials (ΔE_{cat}) include the activation energy and thermodynamic forces required to transfer the electrons from the cathode to the final electron acceptor. These losses are typically significant even with a catalyst. Cathodic overpotentials are typically reduced by selecting a material that does not create high losses, such as platinum, which are often cost-prohibitive. Ohmic over-potentials (ΔE_{Ohm}) include the energy driving the electron and ion flow between the anode and cathode. The electron losses ($\Delta E_{\text{e-}}$) directly correlate with the resistance of the circuit and are quantified by Ohm's law. Ionic losses (ΔE_{ion}) are due to the energy lost moving anions and cations across the membrane. Ionic losses are typically significant and can be reduced by decreasing the distance between the anode, membrane, and cathode as well as internal resistance of the membrane. Ionic losses are one of the easier methods of reducing overall cell overpotentials. Concentration overpotentials (ΔE_{conc}) are caused by the difference between actual and standard conditions at the electrode surfaces. While this can typically be found by using the Nerst equation, the greatest influence is based on the pH gradient between the anode and cathode, each of which are raised to the power of eight, resulting in a high influence on losses. Reducing the pH gradient and introducing alkaline-tolerant species such as *Geothrix* can greatly reduce these overpotentials [5].

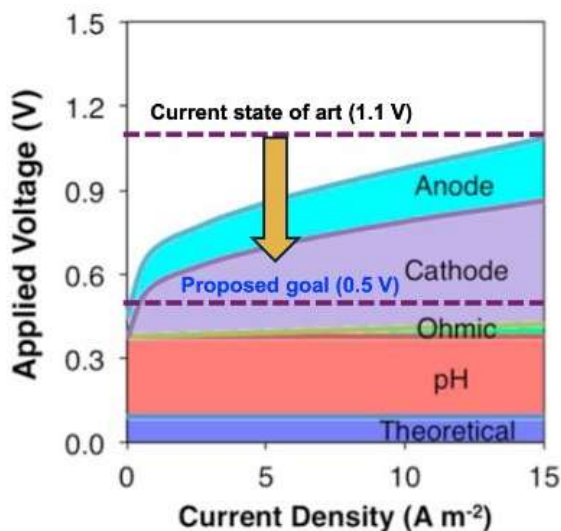


Figure 2: MEC overpotentials as a function of current density (Credit: Dr. Parameswaran)

Currently the applied voltage to achieve high current densities is still very high. The state-of-the-art MEC currently uses approximately 1.1 V to produce 15 A/m^2 , whereas 0.5 V has been proposed as a target development by reduction of overpotentials (Figure 2). Because of this, overpotentials are a key area of importance in the development of MECs as each component and configuration directly contribute to the losses from overpotentials, which affect overall cell efficiency.

1.1.3 Anodes

Anodes are one of the primary areas of study for MECs. As the driving force for the MEC process, maximizing the attachable anode surface area while allowing for uninhibited electron transfer and structural integrity is an apparent area of development. Traditionally, carbonaceous materials are favored in microbial electrochemical platforms due to attachability and conductivity as well as ease of synthesis and economic footprint. Alternatives to traditional materials can include metal/metal oxide-based electrodes, conductive polymers, and thermally reduced/pyrolyzed nitrogen-enriched materials; however, these are far less common [15]. Despite the infrequent usage of non-carbonaceous anodes, titanium foil and nickel plate anodes have been shown to produce

current densities as high as 44.3 A/m² [7], [8]. Carbon anodes, which are the dominant anode material of choice, have typically achieved values ranging from 5 to 20 A/m² with the greatest reported current density as 22.3 A/m² [10]. In material selection for optimal current generation, conductivity, durability, and ARB attachability are key performance parameters, while environmental sustainability and cost are other parameters for consideration.

Geometry is another area of differentiation in anode configuration. MECs have primarily used geometries such as felts, fibers, brushes, and cloths [10], [13], [16], [17]. Granules were also used in some studies to attempt to increase surface area [9]. The goal of these configurations is to maximize attachment area per volume and increase the attachability for ARB. Due to the often-microscopic scale of geometric surface differences, electrode surface areas are difficult to measure, especially with regards to porous and fibrous materials [15]. Because of the difficulty of quantifying surface area, studies typically use projected surface areas, which tends to overestimate current per unit area but allow for ease in calculating scalability.

Lastly, microbial adhesion to the material and durability are factors that are also greatly affected by the anode material. While carbon-based anodes tend to have lower mechanical strength and higher electrical resistance, they are often cheaper, have increased attachability, and are frequently able to be more sustainably sourced. Metal/metal oxide anodes have poor corrosion resistance and reduced attachability but are highly conductive and can reduce losses. Polymer based anodes risk shedding and decreased longevity but can have high attachability and maximize surface area. Nitrogen-enriched carbon anodes have poor structural stability but increase microbial growth. Carbon nanotubes and graphene are typically selected due to their structural strength, conductivity and surface area but can also be costly [15]. Future applications will likely continue to use carbon

nanotubes and graphene anodes until other anodes are able to compete with the superior conductivity, durability, and surface area.

1.1.4 Cathodes

Cathodes are another leading area of development in microbial electrochemistry. The key area of interest in cathode development is material. While platinum is widely considered a highly efficient cathode material due to its superior low cell potential, it is errored by its high capital cost and susceptibility to sulfide poisoning. Thus far cathodes have utilized materials such as stainless steel, titanium, platinum, and nickel alloys, with the largest reported hydrogen production rate being a titanium foil cathode that produced rates of 72 L/L_{cathode}/d at a coulombic efficiency of 96% [8]. Another study utilizing two continuous flow flat-plate style MEC with Ni foam and cobalt-molybdenum coated cathodes was able to produce 50 L/L_{cathode}/d but coulombic efficiencies were not reported [10]. To produce gas at high yields, overpotentials due to cathodes must be minimized. While material is a greatly studied area, there is limited research on the configuration and geometries of cathodes in MECs. Unlike anodes, attachability is not a necessary trait for successful hydrogen production. Instead, redox reactions drive the cathodic reactions, therefore material and surface area for bulk liquid contacting are more important in microbial electrolysis [5]. Some innovation in this area has been shown with modifications to the catholyte and cathode chambers, but very little has occurred to the cathode itself.

1.1.5 Membranes

As described in previous sections, ion exchange membranes are frequently utilized for the liquid separation of the anode and cathode. These membranes can be categorized as anion exchange membrane (AEM), cation exchange membrane (CEM), and bipolar membranes (BPM); however, CEMs and AEMs are primarily used in MECs. They are all made of synthetic material such as

PTFE and are embedded with charged functional groups to select for the types of ions that can pass through them. AEMs are embedded with fixed positive charges such NH_4^+ , NR_3^+ , NR_2H^+ , NHR_2^+ , SR_2^+ , and PR_3^+ , allowing negatively charged ions, whereas CEMs are embedded with negative charges, typically sulfonate groups [18].

Beyond AEMs and CEMs, these membranes have many properties that influence their performance and cost. Ion exchange capacity (IEC) is an especially important parameter that indicates the number of available functional groups that facilitate ion transfer across the membrane. A high IEC equates to a better ion flow rate. Oxygen intrusion, which measures the ability for oxygen to cross the membrane, is another parameter that influences performance. Since most ARB are anaerobes, oxygen can cause major inefficiencies and buildup of aerobes that will dominate the system as well as cause potential shortages in the cell. Other important parameters include water uptake (the amount of water retained during hydration), proton conductivity (the degree to which protons can flow through the membrane), extra ion transport (the selectivity towards the targeted ion), pH splitting (the tendency for pH gradient in the cell to increase, damaging the membrane and performance), and substrate crossover (the ability of large organic molecules to pass through the membrane, causing short circuiting). While not a property of an ion-exchange membrane, membrane fouling is important to consider in MEC design and performance. Buildup of organic and inorganic material on the surface of the membrane can reduce the performance as well as damage the membrane [5]. MECs primarily have utilized AEMs [8], [9], [10], [11], [12], [13], [19].

1.1.6 Performance Metrics

As a diverse family of technologies, it is important to have common performance metrics to compare system efficiency and yield. In MECs the key performance metrics include current

density, coulombic efficiency, coulombic recovery, hydrogen production, hydrogen yield, hydrogen recovery and net energy recovery. Current density (j) is a measure of the electrons produced by ARB utilization of the substrate normalized to the anode surface area or reactor volume (A/m^2 or A/m^3). This is an important measure of the biofilm utilization efficiency as well as helpful in choosing options for scalability. Coulombic efficiency (CE) is the measure of the transmitted current per electron equivalent of COD removed. Coulombic efficiency is calculated with the following equation.

$$CE = 100\% * \left(\frac{IF}{Q\Delta COD} \right)$$

Where I is total current produced, F is Faraday's constant, and Q is the substrate volumetric flowrate. This is the primary parameter for cell efficiency since it shows the ability for the configuration to capture electrons for use in cathodic reactions. The highest reported current density was by Rossi et al. at $44.3 \pm 0.5 A/m^2$. Coulombic recovery (CR) is another important parameter when specific substrate data is not readily available and describes the number of electrons captured versus the electron equivalent of the entirety of the fed substrate and is calculated as

$$CR = 100\% * \left(\frac{IF}{Q * COD_{inf}} \right)$$

Where I is the total current produced, F is Faraday's constant ($F = 96285 \frac{coulombs}{mol e^-}$), Q is the substrate volumetric flowrate, and COD_{inf} is the influent COD. Coulombic recovery is not frequently reported; however, it can be found by multiplying the CE of the system by the substrate removal efficiency.

Hydrogen production rate is the most pertinent metric when assessing the production from a MEC as it indicates the amount of hydrogen gas produced normalized to the cell characteristics. This is

commonly expressed as volume H₂ per volume of the cathode per day or as hydrogen yield which is expressed as mass H₂ per mass of COD consumed or mole H₂ produced per mol of COD consumed [5]. Alternatively, hydrogen production can be described by cathodic hydrogen recovery (r_{cat}), which quantifies how much of the current produced by the cell was converted into hydrogen. The formula to calculate r_{cat} is given as:

$$r_{cat} = 100\% * \frac{V_{gas} * C_{H_2}}{\frac{\int It}{F} * V_{mol}}$$

Where V_{gas} is the volume of gas in L produced in the cathode, C_{H_2} is the concentration of hydrogen in the cathode headspace, I is the current produced, t is time, F is Faraday's constant, and V_{mol} is the molar volume of gas (22.4 L/mol at 20°C or 24.9 L/mol at 30°C) [20].

Net energy recovery (E_{net}) is the measure of the energy output (E_{output}) compared to the applied potential (E_{input}). E_{input} is greatly influenced by overpotentials that increase the required energy to complete electrolysis. This is a key parameter regarding environmental impact due to the emphasis of being a low-energy intensive platform. Net energy recovery is calculated by the following equation.

$$E_{net} = 100\% * \left(\frac{E_{output}}{E_{input}} \right)$$

While not a direct performance metric, transport limitations are an essential component to MECs. At high current densities, the rate of proton diffusion out of the biofilm can be limiting due to a typically neutral bulk liquid (~pH 7), limiting EET [5]. Increasing the buffering capacity, measured as alkalinity, of the anode bulk liquid can increase the rate of diffusion of protons out of the biofilm. Carbonate buffer demonstrates 1.5 to 2 times greater current density than phosphate buffer, due to the higher diffusion coefficient of HCO₃⁻ than H₂PO₄⁻ (1.02 vs 0.83 cm²/d) [21]. In wastewater fed systems, alkalinity is an important metric to consider for compatibility since some waste streams,

such as domestic wastewater, typically have an alkalinity of less than 500 mg/L CaCO₃, limiting proton transfer. Higher alkalinity waste-streams such as animal manure, anaerobic digester liquor, and landfill leachate are more feasible for microbial electrochemical cell feed stock [5].

1.1.7 Cyclic Voltammetry

Cyclic voltammetry (CV) scans are a powerful tool used to analyze the characteristics of biofilms. The basic process of a CV is the cycling of the applied potential of the working electrode at a specified rate and plotting against the current produced, resulting in voltammograms, which can describe the redox properties of the biofilm. There are both turnover and non-turnover CVs. A turnover CV is conducted when there is an electron donor present, indicating the behavior of a bioelectrically active biofilm that is transferring electrons to the anode. A non-turnover CV is conducted in the absence of an electron donor or at very low concentrations so that the biofilm is effectively inactive. This corresponds to the intrinsic redox properties of the biofilm rather than the bioelectrocatalytic behavior of the biofilm. CV scans are typically plotted as current or current density versus applied potential. Turnover CV plots typically produce an S-curve where the inflection point describes the formal potential of the electron transfer site. Non-turnover CVs typically produce peaks correlating to redox-active sites in the biofilm. The analysis of these peaks provides insights such as formal potential or other electron transfer mediators. Non-turnover CVs are valuable to understanding the fundamental electron transfer mechanisms [22]. An alternative to plotting current against applied potential is to create a derivative CV plot where the change in current over change in potential is plotted against applied potential to create more defined peaks at active centers and redox centers. Active centers for ARB can vary, indicating different enzymes and electrons transport methods for. *Geobacter*, a very prevalent ARB in microbial electrochemistry, typically has an active center around -0.15 V vs SHE for wild type strains, but

can even vary even within the same species (-0.22 to +0.09 V) [23]. This means that, while active centers can describe the behavior and optimal conditions of the biofilm, they cannot be used to reliably identify the microbial communities. Despite this, *G. sulfurreducens* has been thoroughly studied and generally has an expected E_{ka} of -0.16 V vs SHE [24]. The active center potential (E_{ka}) can be calculated by taking the midpoint between the potential at which a local maximum current density occurs (E_{ox}) and the potential at which the corresponding local minimum current density occurs (E_{red}) for a potential active center. The equation for this can be shown by the following.

$$E_{ka} = \frac{E_{ox} + E_{red}}{2}$$

1.1.8 Previous Studies

Thus far, very few successful pilot scales have been reported. One of the two pilot MECs utilized winery wastewater in a modular series of single-cell reactors totaling 1000 L; however, this setup was plagued by methanogenesis utilizing the produced electrons producing minimal current density and hydrogen production, 1.01 A/m² and 0.19±0.04 L/L_{cathode}/d respectively [25]. A more recent pilot scale setup was more successful and used post-hydrothermal liquefaction wastewater (PHW) as a substrate. It had an approximate total anode volume of 690 L across four modules, housing carbon brush anodes and stainless-steel wire cloth cathodes. It impressively yielded a maximum current density of 9.3 A/m² from PHW with a hydrogen production rate of 0.5 L/L_{Cathode}/d [20]. Other setups have primarily used bench-scale reactors and have yielded greater results. Set-ups for bench scale systems have differed greatly, but generally are single-chamber, double chamber, or a version of a flat-plate reactor and have utilized a variety of substrates as shown in Table 1 and Table 2. Table 1 describes only studies that utilize acetate as a substrate or other simple substrate systems. Simple substrate systems have historically had better performance than complex substrates achieving current densities as high as 44.3±0.5 A/m², hydrogen yields of

3.95 mol H₂/mol COD (0.498 g H₂/g COD) and volumetric production rates of 72 L/L_{cathode}/d [8], [9]. As advancements in MEC platforms progress, complex substrate systems are gaining traction, and while not yet competing with acetate, have been able to achieve current densities as high as 16.6 A/m², hydrogen yields of 1.18 mol H₂/mol COD (0.587 g H₂/g COD), and volumetric production rates of 9.35±1.73 L/L_{cathode}/d [19], [20], [26]. Cathodic hydrogen recoveries for MECs (r_{cat}) vary greatly and are generally independent of substrate type with simple substrates ranging from 17% to 91% [9], [12]. For complex substrate MECs, recoveries also varies from 50.4% to over 90% [19], [20]. In the comparative study of simple substrates, greater than 89% cathodic hydrogen recovery was maintained across all substrates [12]. In complex substrates, studies have utilized post-hydrothermal liquefaction wastewater (PHW), winery wastewater, food waste, raw domestic wastewater, primary sludge fermentation liquor, corn straw hydrolysate, synthetic wastewater, and switchgrass pyrolysate ranging in COD concentration from 185±20 mg/L 129.4±7.7 g/L [6], [11], [14], [19], [20], [25], [26]. No studies were found that discussed the use of swine wastewater or other animal waste feedstocks.

Table 1: Summary of acetate/simple substrate microbial electrolysis cell studies

Author	Feedstock	MEC Type	Membrane	Anode	Cathode	Applied Voltage (V)	CE (%)	Hydrogen Production	Current Density (A/m ²)
[8]	2 g/L Acetate	2 Chamber	AEM	Titanium foil	Titanium foil	1.1	96±1	72±2 L/L _{cathode} /d	44.3±0.5
[10]	2.72 g/L Acetate	Flat-plate	AEM	Graphite felt	Ni foam	1	-	50 L/L _{cathode} /d	22.8±0.1
[11]	25 mM Acetate	2 Chamber	AEM	Carbon fiber	-	0.85	-	-	18±2
[12]	Acetate	2 Chamber	AEM	Carbon felt	Pt plated titanium	0.8	105	1.92 mol H ₂ /mol COD	7.9
	Ethanol						71	1.38 mol H ₂ /mol COD	7
	Lactate						98	1.56 mol H ₂ /mol COD	7.1
	Propionate						89	1.46 mol H ₂ /mol COD	4.8
	Butyrate						87	1.43 mol H ₂ /mol COD	4.1
	Mixture						83	1.42 mol H ₂ /mol COD	6.9
[9]	1 g/L Acetate	2 Chamber	AEM	Graphite granules	Carbon cloth/Pt	0.13	0.8	3.95 mol H ₂ /mol COD	-
[7]	Acetate	2 Chamber	PEM	Nickel plate	Ni-Co-P deposited on SS316	0.6	96.9±2	4.2±0.5 L/L _{cathode} /d 2.96±2 mol H ₂ /mol COD	310±5 Am ⁻³
[14]	25 mM Acetate	Flat-plate	CEM	Carbon fiber	Stainless steel mesh	-0.4	-	-	10.4±0.9
[13]	Acetate	2 Chamber	AEM	Graphite fiber brushes	Carbon cloth	0.7	95±8	-	4.25±0.2 3
	Propionate					0.7	68±9.5	-	2.7±0.28

Table 2: Summary of complex substrate microbial electrolysis cell studies

Author	Feedstock	MEC Type	Membrane	Anode	Cathode	Applied Voltage (V)	CE (%)	Hydrogen Production	Current Density (A/m ²)
[20]	Synthetic Wastewater	Modular 2 Chamber Pilot	CEM	Carbon brushes	Stainless Steel Mesh	-	-	1.1 L/L _{cathode} /d	16.6
	PHW					-	105%	0.5 L/L _{cathode} /d	9.3
[25]	Winery Wastewater	Modular 1 Chamber Pilot	No membrane	Carbon brushes	Stainless steel mesh	-	-	0.19±0.04 L/L _{cathode} /d	7.3 A/m ³
[19]	Food Waste	2 Chamber	AEM	Carbon felt	Pt plated titanium	0.8	88.9	1.18 mol H ₂ /mol COD	-
[11]	Raw Domestic Wastewater	2 Chamber	AEM	Carbon fiber	-	-	-	-	0.5±0.1
[14]	PS Fermentation Liquor	Flat-plate	CEM	Carbon fiber	Stainless steel mesh	-0.4	-	-	6.4
[6]	Acetate, Corn Straw Hydrolysate	1 Chamber	No membrane	Carbon fiber brush	Pt/C-coated carbon cloth	0.8	74.5	39.7 m L/L _{cathode} /d	9.46±1.5
	Xylose, Corn Straw Hydrolysate	1 Chamber	No membrane	Carbon fiber brush	Pt/C-coated carbon cloth	0.8	71.88 ±2.53	135 m L/L _{cathode} /d	7.68±0.79
[17]	Fermentation Effluent	1 Chamber	No membrane	Carbon cloth	Carbon cloth with Pt catalyst	0.5-1.1	21.3	0.245 L/L _{cathode} /d 0.00489 mol H ₂ /mol COD	-
[26]	Switchgrass Pyrolysate	2 Chamber	PEM	Carbon felt	Pt/carbon	-0.2	66.9±12.69	9.35±1.73 L/L _{cathode} /d	8.76±1.54

1.2 Thesis Structure and Research Objectives

The focus of this thesis is to discuss the development and performance of a novel flat-plate microbial electrolysis cell fed with acetate then swine wastewater as a substrate. This focus forms the following research objectives:

Objective 1: Establish a performance benchmark for the novel flat-plate MEC using acetate under both batch and continuous feeding conditions. This aims to maximize current density and achieve high cathodic conversion efficiency for hydrogen production.

Objective 2: Evaluate the viability of swine wastewater as a substrate in the novel MEC by analyzing current density and hydrogen conversion. Additionally, assess the compatibility and functionality of anode-respiring bacterial communities through cyclic voltammetry analysis.

The thesis is divided into three chapters addressing these objectives with one combined references section at the end of Chapter 3. Chapter 1 introduced the context for the research and literature review for the studies discussed in Chapter 2 and Chapter 3. Chapter 2 details the design and development of the novel microbial electrolysis cell and the resulting experimental setup used throughout the thesis. Chapter 2 then presents the current density and hydrogen performance results from acetate-fed operation under both batch and continuous feed regimes, addressing **Objective 1**. Chapter 2 will also discuss the enzymatic behavior and electrochemical characteristics of the acetate-enriched biofilm to provide the baseline behavior to be used to complete **Objective 2**. Chapter 3 will complete the remainder of **Objective 2** by presenting the platform performance after the switch to batch feed operation with swine wastewater and subsequent cyclic voltammetry analysis to confirm the consistency of the electrochemical behavior of microbial communities in comparison to those characterized in Chapter 2.

Chapter 2 - Acetate Fed MEC Performance

2.1 Introduction

As microbial electrochemical systems continue to evolve, their potential to harness diverse waste streams for resource recovery has gained increased attention. While acetate is less widely available than many of the complex feedstocks being studied, it remains the most commonly used electron donor to advance MEC fundamentals. As one of the simplest and most bioavailable substrates, acetate is highly favored by ARB, promoting biofilm growth while achieving high coulombic recovery. It has been well documented that acetate-fed MECs produce the highest current densities and hydrogen production while having fewer inefficiencies than with complex organics, such as wastewater [8], [10]. This establishes acetate as a benchmark for evaluating the performance of MEC configurations, frequently leading researchers to start up MEC systems with acetate to assess the maximum performance of the system prior to converting to a more complex feedstock. Acetate studies have produced a wide range of current densities ranging from 4 A/m² to values as high as 44.3±0.5 A/m², generally producing 10-20 A/m² [7], [8], [9], [10], [11], [12], [13]. The largest reported value for acetate hydrogen yield was 3.95 mol H₂/mol COD using a platinum coated cathode and 1 g/L acetate [7]. Hydrogen production rate varied greatly by study, but the highest values reported were 50 L/L_{Cathode}/d and 72 L/L_{Cathode}/d utilizing nickel foam and titanium foil cathodes respectively. [8], [10].

Other simple substrates are reported less, but in literature that was reviewed, propionate was the second most reported substrate due to its bioavailability and similarity to acetate, having only one additional carbon in its structure [12], [13]. In a comparative study studying several substrates including ethanol, lactate, propionate, and butyrate, it was found that the corresponding current densities were 89%, 90%, 64%, and 52% of the value achieved by acetate respectively, suggesting

that current density decreases with substrate complexity [13]. Hydrogen yield followed a similar trend in this study, increasing the hydrogen yield from 1.43 mol H₂/mol COD for butyrate to 1.92 mol H₂/mol COD for acetate, with each more complex substrate increasing in yield. The only substrate that did not follow this trend was ethanol, the lone alcohol in the study at 1.38 mol H₂/mol COD [13]. Due to the decreasing performance with substrate complexity, it is important to understand how the system performs under ideal conditions (acetate) prior to introducing a more complex feedstock such as swine wastewater. While acetate has shown strong results in previous literature, feedstocks of greater interest for these systems often are highly complex and come from diverse sources with a wide range of CODs, complexities, and characteristics. However, to assess the performance of a more complex feedstock, acetate must still be used as benchmark to identify the upper limits of the system's performance parameters.

This chapter discusses batch and continuous operation periods utilizing acetate as the sole substrate to achieve four primary objectives: (1) develop a robust ARB biofilm, (2) achieve high current densities that are comparable or greater to existing literature, (3) demonstrate the production of high quality and quantity hydrogen gas, and lastly (4) identify system inefficiencies to prepare for the transition to swine wastewater.

2.2 Improvement from Previous Iterations

The design for this novel flat-plate MEC underwent multiple iterations and design/operational challenges. Although they do not directly relate to the data or conclusions presented in this thesis, it is pertinent that these lessons learned are shared to move towards a better understanding of these technologies and their applications. These changes have had significant impacts on the operational ease of the reactor setup and could be impactful for later scaling.

The first of these many trials involved a very similar cell that was inherited from Dr. Prathap Parameswaran's work at Arizona State University. The cell comprises a plexiglass flat-plate assembly that utilizes a central anode chamber (800 mL), flanked by two cathode chambers (150 mL each). The chambers were separated by anion exchange membranes (AMI, USA), and utilized two titanium plate electron contactors wound with carbon fiber as anodes and stainless-steel mesh as cathodes. Each component was separated by a 1/8" neoprene rubber gasket. An Ag/AgCl reference electrode (BASi, USA) was mounted to the top of the cell to remain in contact with liquid closer to the middle of the anode compartment. The cell was held together by bolts traveling perpendicular to the cell. The anode and cathode chambers were filled with media solution, as described in Sections 2.3.1, at pH 7.2 and 12 respectively. The cathode media selectively excluded the mineral media, FeCl_2 , and $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$ solutions.

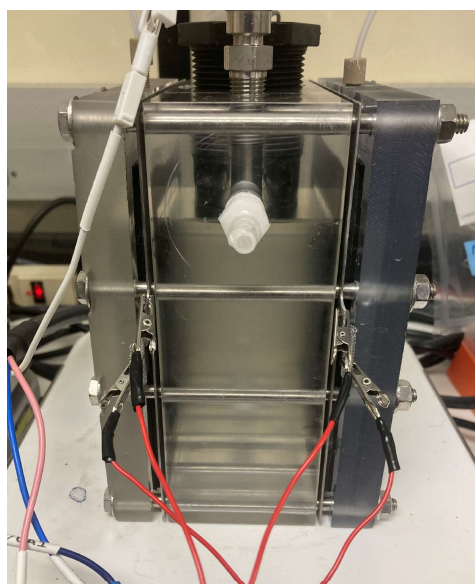
Upon assembly and attempted startup of the reactor, the current immediately spiked and exceeded the safety parameters of the potentiostat and issued an overpotential error. The circuitry and all connections were inspected and there were no obvious reasons leading to the overpotential error. Eventually it was theorized that the bolts which held the anode and other components in alignment were causing short-circuiting between the anode and cathode, reducing the resistance to virtually zero, causing the current to exceed the amperage corresponding to the safety threshold. To combat this, insulating heat shrink wraps were added to each bolt to prevent short circuiting.

This worked for some time; however, additional overpotential errors occurred. After consultation with another researcher, it was theorized that the anode carbon fibers may be touching the membrane which were indeed bowing slightly causing a potential short circuit across the membrane to the cathode. To correct this, a thicker gasket (3/8") was installed between the anode and membranes on either side to prevent direct contact.

After the installation of the larger gasket, the cell struggled to pass a liquid leak test. The bolts were progressively tightened to create a tighter seal. Uneven tightening resulted in stresses in the cathode chamber material cracking the entire component in the middle, as shown in Figure 3a. Later investigation revealed that the outside edge of the gasket was pressing against the inside face of the bolts, causing the gasket to be out of alignment between the face of the layers and could not be detected by visual inspection. After a replacement cathode plate was machined using polycarbonate (Figure 3b), silicone paste was added to help ensure that the cell would not leak while circumventing the need for overtightening.



a)



b)

Figure 3: a) Cracked cathode compartment, b) Overall flat-plate MEC with modified polycarbonate cathode compartment installed (right)

Once an electrically stable, leak proof MEC was established, further issues ensued. A crack occurred at the top of the recently replaced polycarbonate cathode; however, liquid was still holding, and due to lost time, the operation continued with this defect. At this point, the MEC was operated in batch mode with 50 mM acetate, in the same manner described in Section 2.3.2. Gas

production was excellent; however, despite liberal amounts of silicone sealant, the cracked cathode was only able to capture a small fraction of the gas produced on the other anode.

The cracked cathode component was once again machined, installed with an appropriate and uniform tightness of insulated bolts resulting in no cracks, along with ample utilization of silicone sealant (Figure 4). At this point, the MEC held a seal and was able to sustain a stable current density. After this maintained for a few days, the anode compartment was inoculated with scraped biofilm from a previously operating felt anode from a 2-chamber swine wastewater MEC; however, within a few months, transients from the laboratory electric power outlet led to the complete failure of the lab's potentiostat, which took about 3.5 months to complete. This was remedied with an ample inspection of the circuitry and the installation of an uninterrupted power supply equipped with a transient filter. During the studies discussed in Chapter 2 and Chapter 3, it was identified that the sampling port on each cathode posed difficulty due to a lack of pressure relief during sampling, so a pulsed method of removing catholyte was used where the syringe was filled with nitrogen gas, and catholyte was incrementally removed and displaced in the cathode compartment with nitrogen gas.

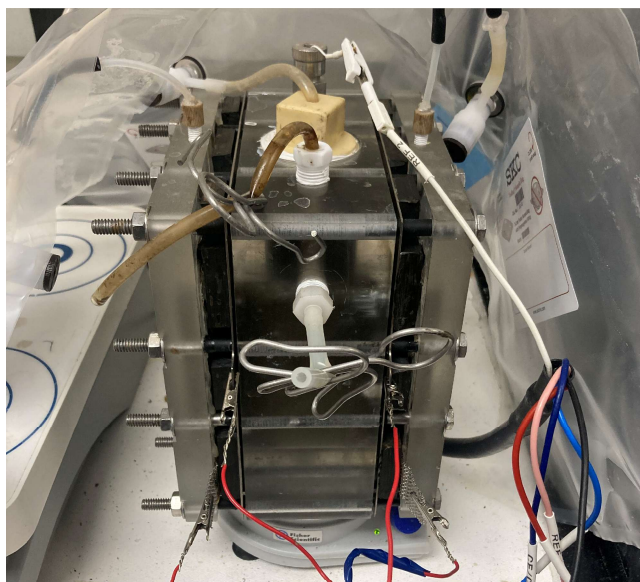


Figure 4: Final iteration of novel flat-plate MEC during swine wastewater batch operation

These lessons learned from the engineering and design deficiencies ultimately led to a robust and resilient flat-plate and potentiostat setup that was used in the studies described in Chapters 2 and 3. To say that it has been tested rigorously would be an understatement. Through ample troubleshooting, hours of staring at the cell, and the unwavering support of Dr. Parameswaran, the following studies were completed.

2.3 Materials and Methods

2.3.1 Novel Flat-Plate Microbial Electrolysis Cell (MEC) Configuration

A flat-plate microbial electrolysis cell was assembled with a shared 800 mL anode chamber, positioned between two 150 mL cathode chambers. The anode and cathode chambers were separated by two anion exchange membranes (AMI, Membranes International, USA) (Figure 6c), which were soaked in 3 M NaCl solution for 48 hours prior to use in the MEC to facilitate the activation of the ion exchange pores on the membranes, as per manufacturer recommendations. The overall arrangement with each MEC component is shown in the exploded view (Figure 5). The anode chamber housed two anode electrodes (Figure 6a), each consisting of a titanium current

/collector plate wound with 24k carbon fiber thread (Fibre Glast, USA). To remove coatings and surface contaminants from the carbon fibers, the anodes were soaked in 1 M nitric acid for three hours followed by twelve hours in 1 M acetate and three hours in 1 M ethanol. The treated anode was then rinsed with deionized water and installed in the cell [27]. The anodes each utilized 182 inches of carbon fiber thread, equating to a combined projected area of 300 cm². This projection assumed that each filament was 6-7 μm in diameter, treating the bundle as a single filament. The combined area of each filament was then used to find the estimated circumference of the bundle and multiplied by the length of the thread. A stainless-steel mesh cathode was used in each cathode (Figure 6b). Each layer was separated by neoprene rubber gaskets to create a gas tight seal and prevent conductive components from touching as shown in the exploded view in Figure 5. A 3/8-inch gasket was placed between the anode and the anion exchange membrane to avoid them from contacting, which can cause short-circuiting. Silicone paste was added between each layer to achieve a complete seal. Gas bags were connected to the headspaces of each chamber to collect the gas produced from each.

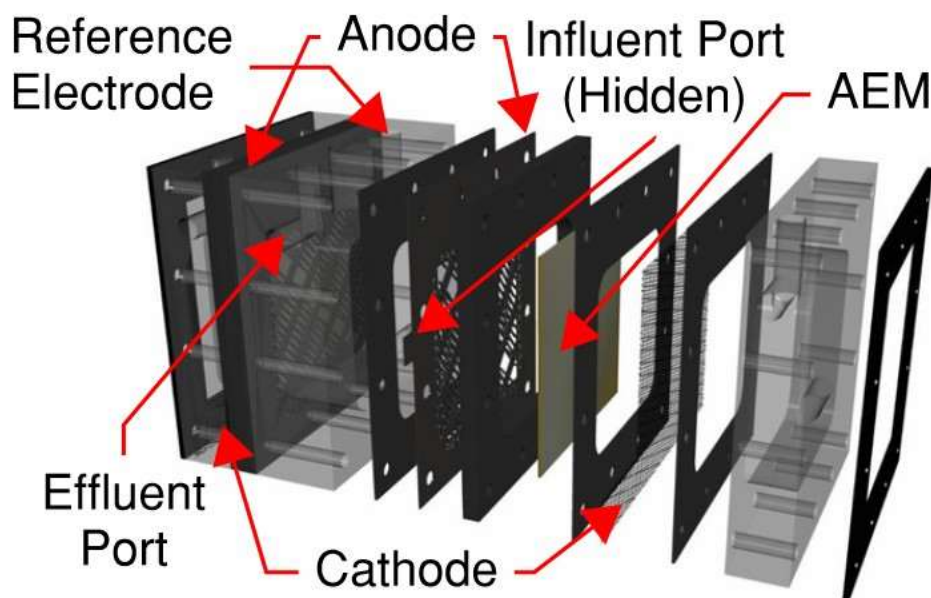
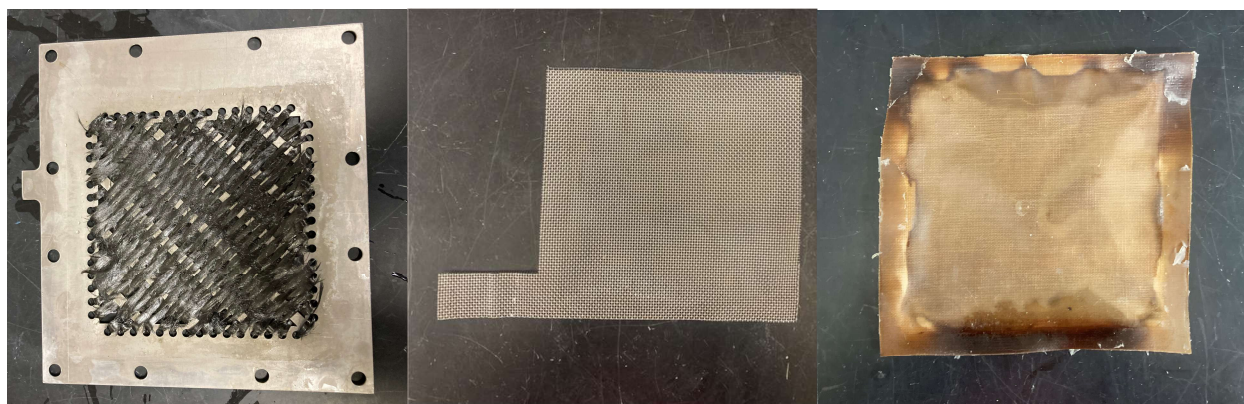


Figure 5: Exploded view of flat-plate microbial electrolysis cell



a) Constructed carbon fiber wound titanium anode, b) Stainless-steel mesh cathode, c) Anion exchange membrane from previous iterations

The anolyte in this setup was a phosphate buffer containing 12.04 g/L Na_2HPO_4 , 2.048 g/L KH_2PO_4 for a combined buffer concentration of 100 mM, 0.41 g/L NH_4Cl , 5 mL of mineral media per 1 L of phosphate buffer containing 0.5 g/L EDTA, 0.082 g/L $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, 0.114 g/L $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.01 g/L H_3BO_3 , 0.02 g/L $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$, 0.001 g/L Na_2SeO_3 , 0.01 g/L $\text{Na}_2\text{WO}_4 \cdot 2\text{H}_2\text{O}$, 0.02 g/L $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$, 1.16 g/L MgCl_2 , 0.59 g/L $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 0.05 g/L ZnCl_2 , 0.01 g/L $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 0.01 g/L $\text{AlK}(\text{SO}_4)_2$ [28]. Additionally, 4 g/L FeCl_2 stock solution and 37.2 g/L $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$ stock solution were added under anaerobic conditions [29]. This anode media was set to a pH of 7.2 then sparged with 99.999% ultra-high purity nitrogen to remove any dissolved oxygen. The catholyte for this setup was 100 mM NaCl solution set to a pH of 11, reducing the pH gradient from the previous iterations as described in Section 2.2. The anode was stirred continuously to reduce any substrate gradient transport limitations at approximately 300 rpm with a magnetic stirrer with analog speed control.

The MEC was connected to a VMP3 Multichannel Potentiostat (Bio-Logic USA, USA) and operated in chronoamperometry mode by poisoning the anode at -300 mV versus an Ag/AgCl reference electrode. Gas production was measured by attaching the headspace of each chamber to

separate 3-liter Tedlar bags. Hydrogen and methane were measured by analyzing duplicate 500 μ L samples with a thermal conductivity detector-equipped gas chromatography (Shimadzu GC-2010 Plus, USA) utilizing argon as a carrier gas at a flow rate of 8.8 mL/min and pressure of 88.8 kPa. The temperature of the injection was 200°C, the temperature of the column was 80°C, and the temperature of the detector was 150°C. The purities of hydrogen and methane were then determined by comparing the areas of the detection peaks to a calibration curve. To quantify the gas produced, a 500 mL syringe was used to remove gas from each Tedlar bag until emptied and the total volume was multiplied by the purities its respective headspace to determine the volumes of hydrogen and methane. The continuous flow operation utilized a high-performance liquid chromatographer (HPLC) (Shimadzu LC-20AT, USA) equipped with photodiode array and refractive index detectors and used an Aminex HPX-87H column (300 mm x 7.8 mm, Bio Rad Corp., USA) to quantify volatile fatty acid concentration in the influent and effluent. The HPLC method used a pump flow rate of 0.65 mL/min, oven temperature of 50°C, and eluent of 2.5 mM sulfuric acid. tCOD and sCOD were measured using HACH TNT 822 kits

2.3.2 Acetate Batch Feeding

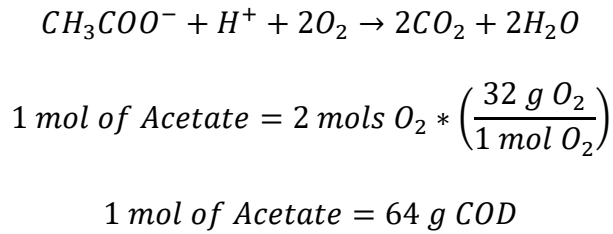
The system described in Section 2.3.1 was fed with seven successive batches of approximately 50 mM acetate solution and allowed to consume the substrate until the current density was below 10% of the maximum current density for a given batch. The first batch cycle included the startup and inoculation of the reactor, utilizing ARB scrapings of a 2 chamber MEC with a carbon felt anode that used swine wastewater at a pH of 7.2 as a substrate and anolyte. The anode chamber of the MEC was first filled approximately halfway with the anolyte recipe from Section 2.3.1. The anode scraping was then added to a vial with approximately 30 mL of anolyte, and the mixture was added to the anode chamber of the novel flat-plate MEC. The remainder of the anode chamber

was then filled with anolyte. After the conclusion of each batch, the bulk liquid was replaced with the anolyte recipe described in Section 2.3.1 under anaerobic conditions within a glove box to replenish the buffer capacity and maintain the experimental conditions for each batch. Headspace composition and total gas produced were measured three times during operation. Since gas quantification and characterization did not occur after each batch, each gas sample contained the cumulative gas produced between each measurement. For the acetate batch experiments, the first measurement contained the gas produced by batch one, the second measurement contained the gas produced between batches two through four, and the third measurement contained the gas produced from batches six and seven. Since accurate acetate concentrations were not determined by HPLC analyses during the batch operation, hydrogen yield calculations conservatively assumed the utilization of the entire substrate between each quantification. If several successive batch cycles elapsed prior to gas quantification, the total cumulative theoretical COD consumption was used to calculate H_2 yields. Both the anode and cathode chamber pH were monitored regularly and adjusted back to 7.2 and 11 with HCl and NaOH respectively with the catholyte being retained and replenished as it was partially consumed by the cathodic hydrogen evolution reaction. Current density and applied voltage were measured every two minutes by the potentiostat.

2.3.3 Acetate Continuous Feeding

Once the batch cycles were completed, the flat-plate MEC anode was fed semi continuously for 32 days with the anolyte discussed in Section 2.3.1 with approximately 20 mM acetate at 1 mL/minute to achieve a hydraulic retention time of approximately 13 hours. During this 32-day period, there was a 14-day gap in operation corresponding to an academic break. A 50 mM acetate spike was added at the beginning of the break to provide food for the biofilm, but the current and data produced during that period was omitted from the data shown in Figure 10 for clarity of the

intention of this study. During continuous operation, two peristaltic pumps (KXP100, Kamoer, China) were attached to a lower anode chamber influent port and an upper anode chamber effluent port as depicted in Figure 5, respectively. The flow rate for these pumps were regularly calibrated to ensure matching flowrates to meet the target retention time. Samples were taken directly from the influent and effluent lines through mid-tubing sampling ports and tested for pH and VFA concentration. Influent and effluent VFA concentrations were converted to COD and used to calculate the COD removal of the platform and corresponding coulombic efficiency. Since only the substrate present in the sample was acetate, the following conversion was utilized to calculate COD.



Corresponding coulombic recoveries were also calculated to allow for comparison with the initial batch studies. The feed container was maintained at anaerobic conditions to minimize contamination and mixed on a magnetic stir plate to maintain homogeneity. As new anolyte was added to the feed container, the mixture was sparged with 99.999% ultra-high purity nitrogen gas and plugged. The headspace of the feed container was attached with a gas bag filled with 99.999% ultra-high purity nitrogen gas to displace the anolyte fed to the MEC. Gas space composition and volumes of gas produced by each chamber were collected more frequently during this period of operation due to increased gas production and to reduce the opportunity for hydrogenotrophic methanogenesis to occur that may have reduced any measured hydrogen production and yields in the acetate batch cycles. Anodic pH was monitored with each influent and effluent sample, but cathodic pH was monitored sparingly due to the limitations discussed in Section 2.2. As the

catholyte was consumed due to cathodic electrolysis, additional 100 NaCl solution at pH 11 was added to refill the volume of the cathode chamber while also diluting a rising pH. Produced current density and applied voltage were measured every two minutes.

2.3.4 Cyclic Voltammetry (CV) Scans

At the conclusion of both the batch and continuous acetate feed operations, turnover and non-turnover CV scans were conducted utilizing the cyclic voltammetry technique within EC-Lab V11.61 (Bio-Logic, USA) program that was also used for chronoamperometry. The anode was initially set at -0.300 versus an Ag/AgCl reference electrode. The scan then varied the anode potential from -0.500 V to 0.100 V at 1 mV/s. These values were converted to potential versus a standard hydrogen electrode by adding +0.242 V to the Ag/AgCl potentials, known from standard electrochemical conversion. The scans were performed in triplicate. The non-turnover CV was completed after the continuous acetate feed operation with fresh anolyte without any substrate. 50 mM acetate was then added, and the turnover CV was completed with the same settings at greater than 90% of the expected peak current density. Non-turnover CVs can reveal the catalytic active centers involved in extracellular electron transfer (EET) of ARB such as *Geobacter*, while turnover CVs can confirm the predominant EET mechanism [30].

2.4 Results and Discussion

2.4.1 Acetate Batch Feeding

The flat plate MEC showed rapid development of a robust biofilm as evidenced by increase in current density, reaching 8.99 A/m² at an applied voltage of 1.32 V, after which sequential batch experiments further reinforced the ARB biofilm development (Figure 7). Although the densities did not achieve the same as the highest acetate range, the typical range for similar systems using comparable anodes is 4-18 A/m² [11], [13]. After the fifth peak, the subsequent two current density

peaks achieved similar current densities, signaling that the biofilm had reached its maximum current density. Since this was a very young biofilm, there was likely still the ability to increase the current produced.

Coulombic recovery for these batches varied greatly during the batch study ranging from 16% to 78% with an average recovery of 47%. The recoveries generally correlated with high current densities as expected, except for batch four which produced a very sharp increase and decline in current, yielding fewer total electrons. This indicates that the substrate utilized during this peak went to products other than electron production. Without HPLC analysis of the substrate, the actual substrate utilization cannot be confirmed for batch four; however, the low current produced indicates that the substrate was largely utilized. This could be caused by contamination of the batch's bulk liquid and presence of competing microbes. The current production returned to expected behavior for the final three batches, indicating that the issues were isolated to only that batch. Utilization otherwise appeared to be high due to the high peaks followed by plateaus at low current production as shown in Figure 7.

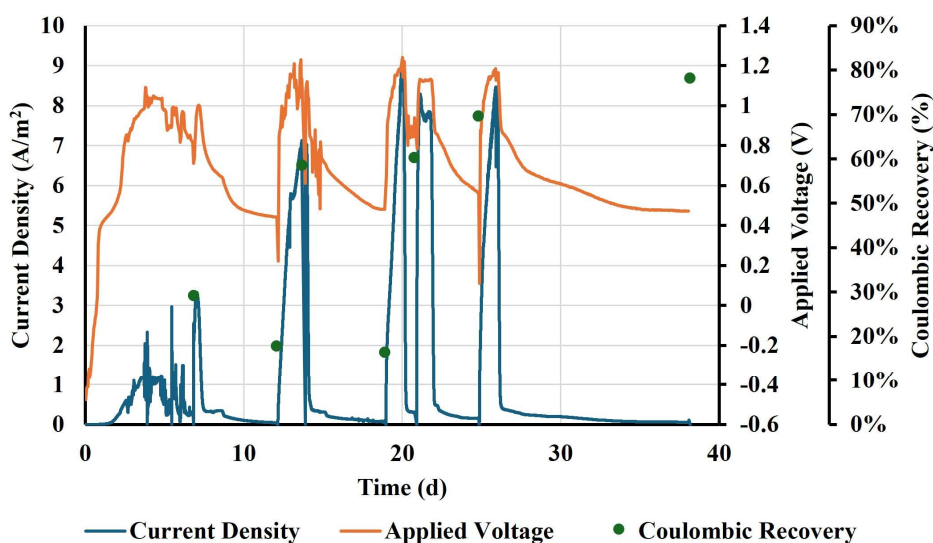


Figure 7: Current density, applied voltage and CR data for acetate batch cycles

Due to qualitative observation of low gas production rate, gas measurements were only measured three times, each time measuring the cumulative volume produced between quantifications. These measurements resulted in a maximum daily cathodic hydrogen production rate of 0.99 L/L_{cathode}/d with a sustained H₂ headspace concentration of 97±6% v/v (Figure 16). This value decreased during each measurement to 0.51 L/L_{cathode}/d then 0.2 L/L_{cathode}/d on the final measurement. Correspondingly, the hydrogen yield dropped from 1.02 to 0.26 mol H₂/mol COD showing low efficiency in converting current to hydrogen. Minimal gas was produced by the anode and primarily consisted of nitrogen, methane, and carbon dioxide at an average rate of 40 mL/L_{cathode}/d. While the volumetric hydrogen production rate observed during this study was substantially lower than the expected production for acetate fed MECs, these values were artificially deflated by the long periods of time between feeds [8]. Although these values would be improved if the time periods between batches were subtracted, the yields and cathodic hydrogen recoveries (r_{cat}) (averages of 0.52 and 34% respectively) still indicate that there was reduced hydrogen produced per mol of COD and most of current that made it to the cathode was not converted to hydrogen.

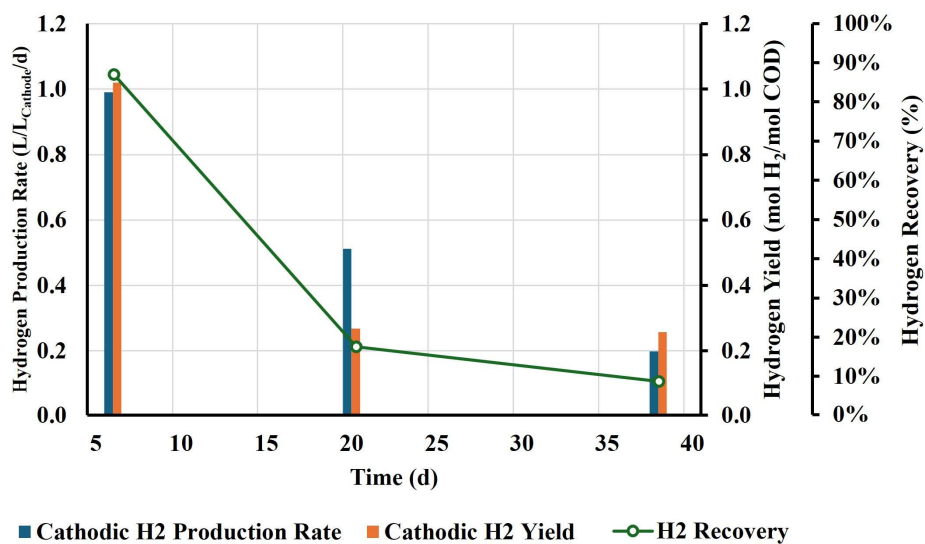


Figure 8: Cathodic H₂ production and yield data for acetate batch cycles

A potential explanation for the observed cell inefficiencies in the cathode was the large changes in cathodic pH during each batch. To reduce ionic overpotentials in the cell while maintaining circuit stability, the anode and cathode pH were set at 7.2 and 11, respectively. Based on knowledge of anodic and cathodic reactions described in Section 1.1.1, a pH gradient of 4-5 units is anticipated to prevail between the anode and cathode compartments even with a well buffered anolyte solution. This is due to the inherent optimal pH conditions for the anode and cathode half reactions. In this study, it was observed that the anode and cathode pH converged on four of the batches, which contradicts the expected behavior of the cell. On three of these occurrences, the pH of the cathode dropped below 8 as shown in Figure 9. Initially, it was hypothesized that mixing of catholyte and anolyte media occurred which could indicate that protons were leaking across the membrane into the cathode; however, the anode and cathode pH diverged during the fifth batch, demonstrating the ability to hold a pH gradient. This suggests that the membrane retained ion selectivity, as a compromised membrane would have resulted in continued convergence. One possible source for cathodic acidification that was reported in literature was chlorine evolution caused by a combination of a high cathodic NaCl concentrations in the cathode compartment and applied voltage. This reaction has been observed in seawater electrolysis, that when high potentials are applied to a highly saline catholyte, HCl and other chlorine species can form in solution, causing cathode acidification [31]. Since the catholyte was originally 100 mM NaCl and the pH was adjusted with HCl and NaOH addition instead of complete replacement of the catholyte, the NaCl produced by the neutralization reaction would have accumulated and approached the conditions of seawater. Since the membrane was deemed to be intact, this issue was not explored further, and greater pH monitoring in each chamber was implemented.

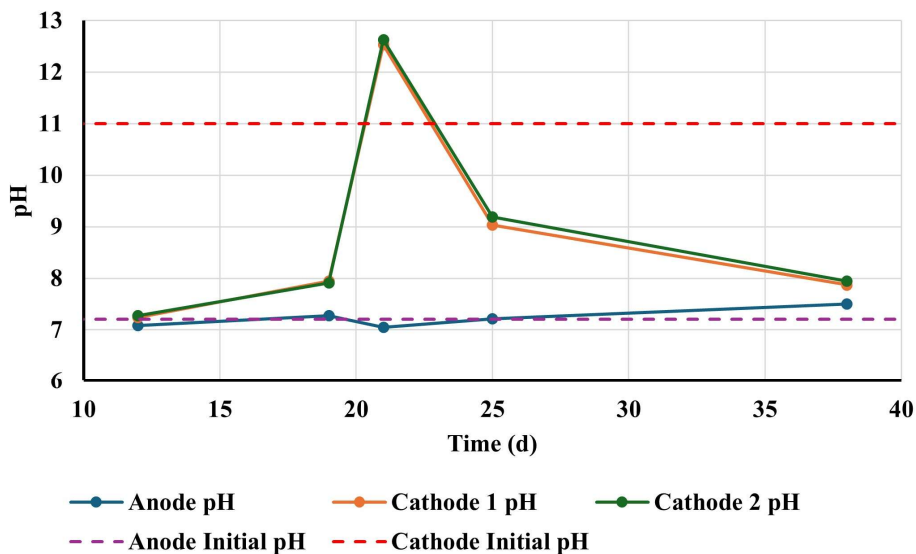


Figure 9: Variation in end of batch cycle pH

The acetate batch study accomplished three of the four objectives set by the study. First, the acetate-fed batch MEC led to the rapid attachment and development of a ARB biofilm. Second, while not exceeding current densities found in literature, this study was able to achieve comparable measurements for carbon-based anodes. Third, multiple sources of physical and operational inefficiencies were identified including frequency of gas measurement, cathodic acidification, and the necessity for catholyte replacement or buffering for extended operation. Due to the inefficiencies and difficulties identified during and after the study, hydrogen production, yield, and recovery were all below the expected range for acetate-fed MEC operation.

Future batch acetate studies should continue to identify strategies to increase current density and gas performance while reducing overpotential losses. Primary areas of improvement are reduction of gasket thickness between the anode and AEM, reduction of the pH gradient through an alkalophilic ARB, and cathodic pH control. These strategies show great promise in improving these inefficiencies in targeting the apparent shortfalls of the design and operating parameters.

Additionally, future studies should increase the sampling frequency of operational parameters and performance data to accomplish more targeted optimization of the platform and reduce data gaps.

2.4.2 Acetate Continuous Feeding

During the continuous operation phase, the system maintained an average current density of 5.84 A/m² at 1.20 V, with a maximum recorded value of 7.31 A/m² at 1.18 V. Similar to the range stated in Section 2.4.1, these current densities fall within the expected range for acetate fed MECs utilizing carbonaceous anodes, although at the lower end of the range. The observed current densities correlated to very high substrate removal efficiencies and subsequent coulombic recoveries. Under continuous flow conditions, the MEC had an average measured acetate removal efficiency of 98.0% and columbic recovery of 94% (average coulombic efficiency of 97.1%). This shows that not only was the substrate almost entirely consumed but the electrons produced were able to be captured as current. As a more developed biofilm fed with a lower substrate concentration, the high substrate removal is also an indication that higher substrate concentrations could produce high current densities under continuous flow operation while still achieving high removal and subsequent efficiencies. Similar to the batch acetate study, the applied voltage generally had a direct correlation to the magnitude of current density as shown in Figure 10.

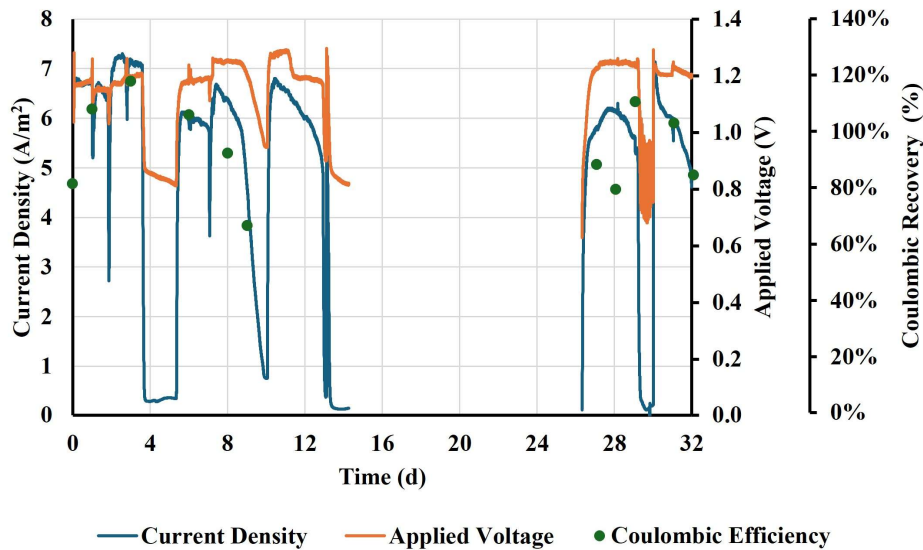


Figure 10: Current density, applied voltage, and coulombic recovery data for acetate continuous feed operation

In comparison to the batch acetate feed, which achieved hydrogen production rates of 0.99 L/L_{cathode}/d and hydrogen yields of 1.02 mol H₂/mol COD, the continuous acetate feed demonstrated a substantial increase to 7.29 L/L_{cathode}/d and 2.56 mol H₂/mol COD, respectively. These improvements are likely due to the high consumption rate of the substrate and corresponding coulombic recoveries. Additionally, the gas produced during this continuous feed operation was very high purity with concentrations of 97±6% v/v. As the theoretical maximum for hydrogen yield is 4 mol H₂/mol COD, 2.56 mol H₂/mol COD is an exciting result. Most acetate and simple substrate systems ranged from 1.38 to 3.95 mol H₂/mol COD with most reported values achieving below 2 mol H₂/mol COD [9], [12]. This shows overall good health of the system as hydrogen yield is a metric that accounts for both anode and cathode performance with substrate removal and hydrogen production, respectively. The continuous feed study also exhibited an increase in hydrogen recovery (average of 50%), demonstrating an increase in the number of electrons produced at the anode being utilized for hydrogen evolution at the cathode. It should be noted that

the decrease in current between day 3 and 5 of the study can be attributed to the feed container holding the influent media and substrate ran dry, creating downtime to make new media. This means that there was less substrate was able to be utilized to drive HER and contributed to the lower hydrogen production rate at day 9 in Figure 11. Cathodic pH was not able to be monitored during continuous operation due to the design limitations discussed in Section 2.2, so it is unknown if any temporary acidification occurred; however, at the conclusion of the study, cathodic pH was measured to be greater than 11, indicating that the overall the process did not result in cathode acidification.

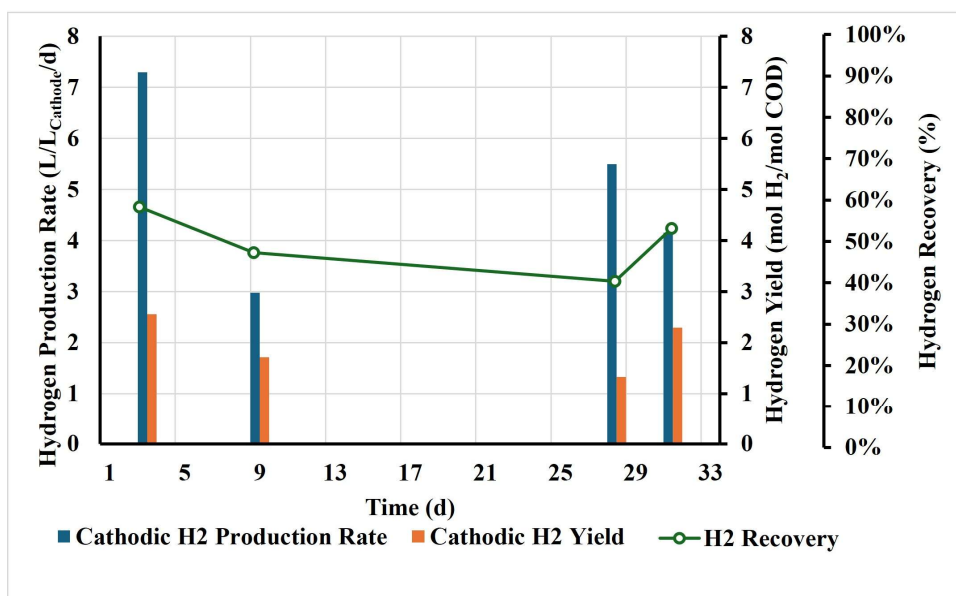


Figure 11: Cathodic H₂ production and yield data for acetate continuous feed operation

This study was able to achieve three of the four objectives of the study. First, continued feed of substrate allowed for greater biofilm development and better preparation for conversion to a new substrate. Second, current densities and corresponding performance data generally fell within the expected ranges in literature for similar setups. Lastly, this study greatly improved gas performance overall with regards to hydrogen production rates (0.57 L/L_{cathode}/d to 4.98 L/L_{cathode}/d), yields (0.52 mol H₂/mol COD to 2.0 mol H₂/mol COD), and cathodic hydrogen

recoveries (37.7% to 49.8%) as well as producing more consistent gas performance (Figure 16). There were no additional inefficiencies identified beyond those specified in Section 2.4.2.

2.4.3 Cyclic Voltammetry Scans

The cyclic voltammetry scans produced at the conclusion of the acetate batch and continuous operations experienced significant hysteresis at the higher ends of the scans, making identification of active centers difficult. Under turnover conditions, one active center was identified below -0.1 V vs SHE where hysteresis was negligible. The oxidation and reduction potentials were identified at -0.141 V and -0.259 V, respectively, equating to an E_{ka} of -0.200 V vs SHE (Figure 12a). The derivative CV (DCV) plot corroborates this active center with a very active peak at -0.216 V vs SHE (Figure 12c). This was determined by taking the average of the local maximum and minimum at the area of interest. To reduce noise in the DCV, these maximums and minimums were taken from a plot of the three-point moving average of the change in current divided by the change in applied potential. There is some activity in the DCV around 0.058 and 0.242 V vs SHE, but the hysteresis in the CV plot makes it difficult to conclusively assign an active center. Non-turnover appears to produce another active center with an E_{ox} and E_{red} at 0.232 V and 0.197 V, respectively, and an E_{ka} of 0.215 V; however, this was likely an abiotic reaction since it was outside of the expected range for ARB substrate consumption and that more current is flowing through the anode at the higher poised potentials (Figure 12c). This is also supported by the derivative CV plot in Figure 12d due to the lack of significant activity at 0.215 V. Although not visible in the non-turnover CV plot, there appears to be some heightened but not well-defined activity around -0.2 V vs SHE, indicating a possible active center, which would additionally support the active center identified by the turnover conditions.

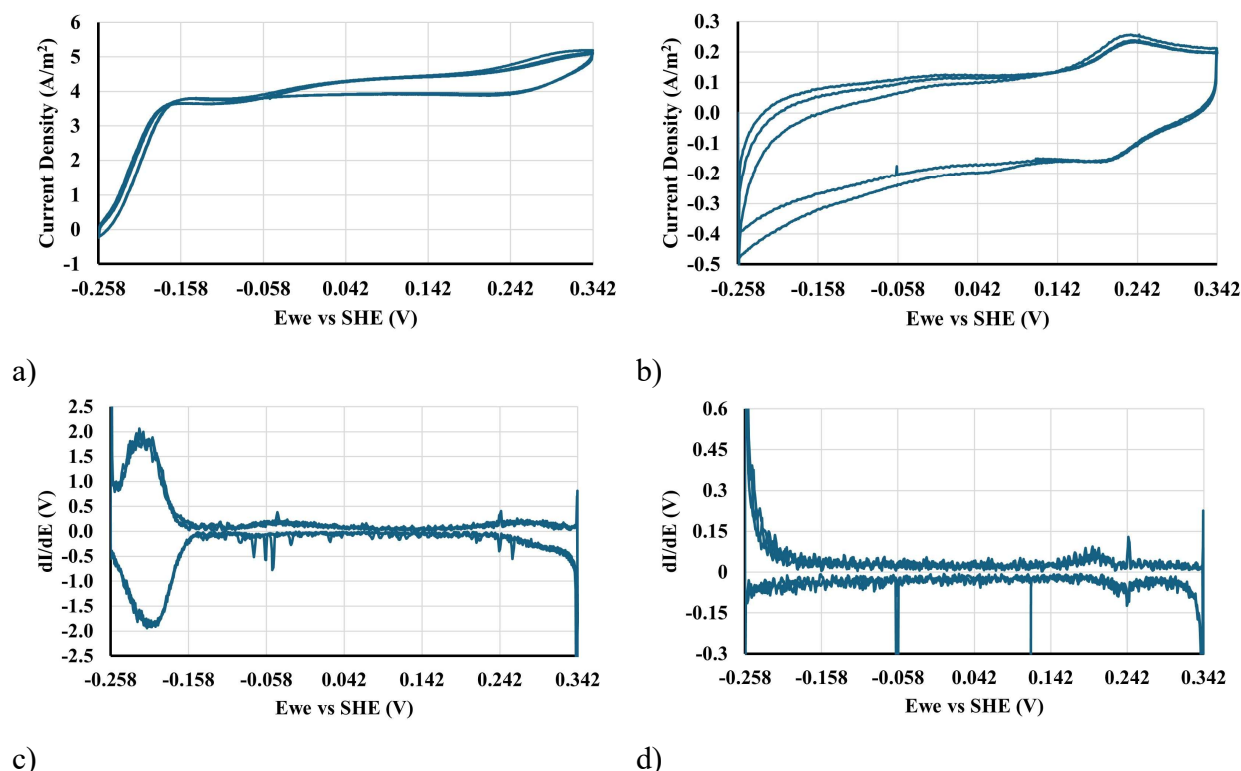


Figure 12: a) Acetate turnover cyclic voltammetry scan, b) Acetate non-turnover cyclic voltammetry scan, c) Acetate turnover derivative cyclic voltammetry scan, d) Acetate derivative cyclic voltammetry scan

The turnover and non-turnover CV scans from the acetate enriched biofilm showed one consistent active center around -0.200 V vs SHE. This was corroborated by heightened activity in three of the four plots, with well defined activity in both turnover plots. In the future, this should be repeated at a lower scan range to try to reduce hysteresis that occurred at higher applied potentials. While this is generally in line with what is expected for *Geobacter* dominated biofilms consuming acetate (-0.16 V vs SHE), the species cannot be definitively determined without DNA analysis [24].

Chapter 3 - Swine Wastewater Fed MEC Performance

3.1 Introduction

Animal manure has gained interest in recent years due to its value proposition as a high strength waste and global abundance. In the United State alone, the U.S. Department of Agriculture estimates that, 335 million dry tons of manure produced each year from animal feed operations and concentrated animal feed operations [32]. Swine waste, a particularly strong waste stream, contains incredibly high COD, total nitrogen, and total phosphorus at 3,000-30,000 mg/L, 210-2100 mg N/L, and 100-620 mg P/L, respectively [33]. Globally in 2023, the Food and Agriculture Organization of the United Nations estimated that there were approximately 965 million head produced globally, and that number continues to rise. The United States accounts for approximately 74.5 million head, ranking third globally in annual hog production behind China and the European Union [34], [35]. Given that hogs produce approximately 0.375 dry tons of manure per hog per year, even a conservative estimate of the cumulative organic and nutrient loads is high both globally and domestically, creating a high value proposition for resource recovery [32]. Currently, the waste from hog operations is largely stored in open lagoons and lead to significant release of gaseous emissions and contamination of water bodies due to runoff and leaching prior to land application [36]. While this land application allows for some recovery of nutrients in crop uptake, the losses to the environment are both harmful and wasteful of a potentially valuable resource. As technology has advanced, and growing pressures to develop sustainable technologies drives research to pursue new avenues of development, a shift needs to occur that recognizes the value proposition of by-products traditionally viewed as waste as valuable sources of materials and energy in order to provide resiliency to supply chains while reducing harmful impacts on ecosystems and dependent economic activity.

MEC systems are a novel and potentially viable biotechnology platform to harness the inherent energy contained in these resources. MECs can be used either as a standalone technology or in combination with conventional treatment platforms such as anaerobic digestion, anaerobic membrane bioreactors, or others. While swine wastewater performance in MECs has not yet been reported in literature, there is a pattern emerging around complex organic feedstocks that are in high abundance and few applications in existing markets, particularly agriculture wastes [6], [26]. These high strength, complex matrix feedstocks have generally performed well, although producing lower current densities and hydrogen production than acetate fed systems and systems fed with simple substrates. This suggests a likely synergistic relationship between this technology and the agriculture sector, strengthening the hypothesis of successful process performance with the MEC platform.

This chapter discusses the batch feed operation of the same novel flat-plate MEC discussed in Chapter 2, using swine wastewater as a substrate. The goals of this study were to: (1) demonstrate stable operation of a swine wastewater MEC, (2) observe current density, applied voltage, and subsequent gas performance data, and (3) compare electroactive signatures of the ARB biofilm fed with swine wastewater with the acetate fed biofilm.

3.2 Materials and Methods

3.2.1 Swine Wastewater Batch Feeding

The same cell configuration described in the Section 2.2.1 was used for batch trials with swine wastewater (COD range of 5000-30,000 mg/L) as a substrate and anolyte. Following the acetate continuous feed operation outlined in Section 2.3.3, the anolyte in the novel flat-plate MEC was replaced with strained swine wastewater, supplemented with solids retained from centrifuging the bulk liquid of a 2-chamber MEC fed with swine wastewater for a period of more than one year.

The rationale was to include the mixed microbial communities pre-enriched for complete degradation of the swine wastewater. Six successive batch cycles fed with swine waste as the electron donor were conducted with the retained solids in the suspension by centrifugation. As the MEC did not have an integral solids separation technology such as a membrane module, the entire bulk liquid had to be removed between each batch. The bulk liquid was centrifuged at 15054 rpm for 20 minutes (Eppendorf 5920 R, Germany) to retain most of the solids, and the supernatant was replaced with new strained swine wastewater. The solids were resuspended, pH adjusted to 7.2 using HCl and NaOH, and sparged with 99.999% ultra-high purity nitrogen gas. Solids analysis, tCOD, sCOD, and pH measurements were taken from bulk liquid before and after each feed. COD samples were quantified with HACH TNT 822 vial tests. Gas measurements were taken at the conclusion of each batch as per methods described in Section 2.3.1.

3.2.2 Cyclic Voltammetry Scans

At the conclusion of successive swine batch MEC cycles, turnover and non-turnover CV scans were conducted utilizing the cyclic voltammetry technique described in Section 2.3.4. The turnover CV scan was completed during the final batch when the current density reached 90% of its expected value. The non-turnover CV was then completed after the batch had completed its peak current density and the tail following the maximum current density had plateaued indicating that minimal consumption of substrate was occurring.

3.3 Results and Discussion

3.3.1 Swine Wastewater Batch Feeding

The swine wastewater batch trials yielded highly promising results. The MEC rapidly acclimated to the new substrate and added solids producing a current density of 4.47 A/m² at 1.31 V and a coulombic recovery of 39% on the first batch, which is within the expected range for complex

substrates. Coulombic efficiency was not reported for this batch due to an error in the COD measurement which stated an increase in COD, which is not possible due to conservation of mass. The subsequent two batches increased with each progressive batch, achieving a maximum peak current density of 10.32 A/m^2 with an applied voltage of 1.35 V and coulombic efficiency of 86%. This current density is very exciting as it was one of the highest reported current densities for real wastewater systems as well as synthetic high strength substrates. The corresponding initial tCOD was $12,905 \text{ mg/L}$ for the peak, with a total solids concentration of 1.07% w/v in the anode compartment. On the fourth batch, the initial tCOD and TS increased substantially to $30,015 \text{ mg/L}$ and 2.3%, respectively, due to a large change in the influent wastewater concentration. This caused an immediate drop in current density and high variability in the applied potential as shown in Figure 13. The fifth batch also had unique behavior as there was not a defined peak but rather increased sharply then declined for the remainder of the batch. The average current density during the swine wastewater batch operation period was 2.47 A/m^2 at an applied voltage of 1.08 V , with an average coulombic efficiency of 51%. Substrate removal was also high given the strength and complexity of the substrate with average tCOD and sCOD removals were 46% and 51%, respectively. Despite the high variability in the initial tCOD and TS concentrations (Figure 14), the peak current density always remained in the expected range for real wastewater systems.

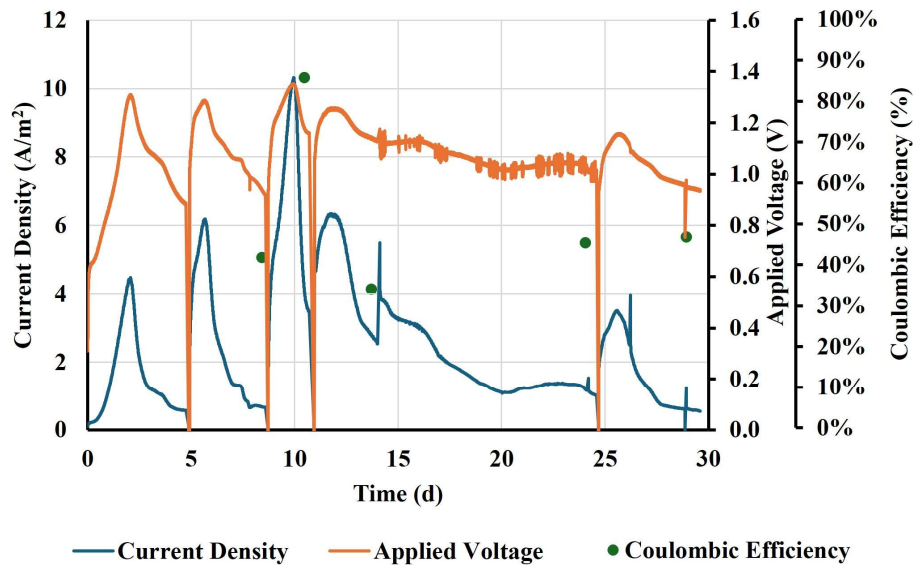


Figure 13: Current density, applied voltage, and coulombic efficiency data for swine batch cycles

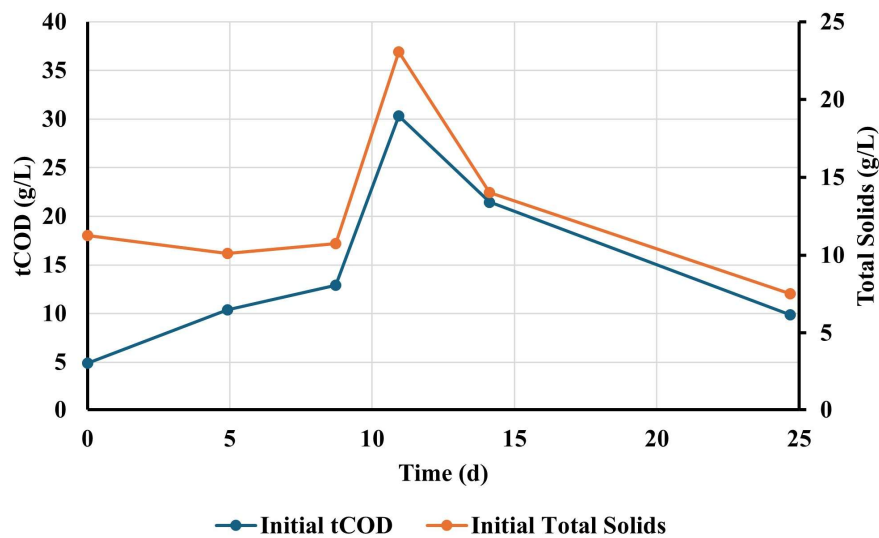


Figure 14: Initial tCOD and total solids concentrations for swine batch cycles

Gas data also behaved as expected for a complex substrate-fed MEC. The maximum H_2 production rate achieved was $3.56 \text{ L/L}_{\text{cathode}}/\text{d}$ and had an average production rate of $1.94 \text{ L/L}_{\text{cathode}}/\text{d}$, whereas the literature reports values ranging from less than $1 \text{ L/L}_{\text{cathode}}/\text{d}$ to $9.35 \pm 1.73 \text{ L/L}_{\text{cathode}}/\text{d}$ [6], [26].

Hydrogen yields reached a maximum of 0.83 mol H₂/mol COD and an average of 0.54 mol H₂/mol COD, which also falls with the expected range of 0.00489 mol H₂/mol COD to 1.18 mol H₂/mol COD for real wastewater systems [17], [19]. It should be noted that yield and recovery were reported far less than production rate, so this range should not be considered exhaustive. Gas production similar to current production followed the characteristics of the initial batch conditions, producing the highest production and yields on the fourth batch, then fall sharply after the tCOD and solids shock to the reactor. After the influent returned to more typical values, the yield steadily increased while the production rate continued to decline for the remainder of the study. This study also demonstrated acceptable hydrogen recovery, reporting values ranging from 21% to 46% with an average hydrogen purity of 95±2% v/v, although still demonstrating room for improvement to achieve the upper range of cathodic hydrogen recovery achieved by comparable systems (~50-90%) [19], [20].

These recoveries show a continuation of the electron losses in the acetate studies. The overall range of r_{cat} across all studies was 8.9-86.9% with the average measured recovery being 38% (Figure 16). While this falls within the reported range for MEC recoveries, an average of 38% still means that over half of the electrons produced by substrate utilization were not converted to hydrogen. These losses are likely due to two factors. The first is electron losses to competing reactions in the cathode. The second is cathode selectivity for hydrogen evolution reaction. Stainless steel has historically been used due to its low cost and ease but may not be the ideal catalyst for the reaction to proceed most efficiently.

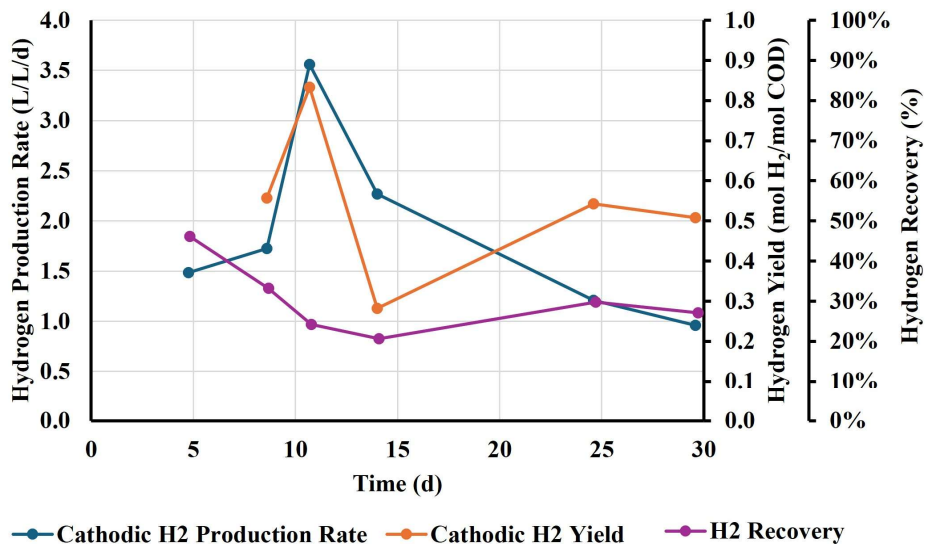


Figure 15: Cathodic H₂ production and yield data for swine batch cycles

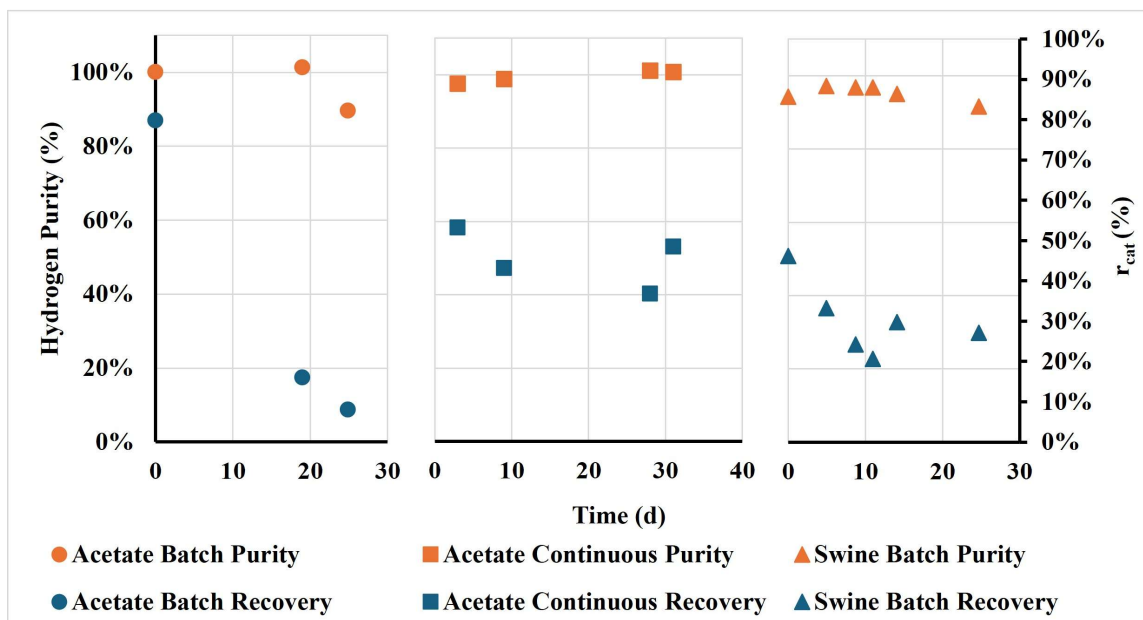


Figure 16: Combined cathodic H₂ production and yield data

Unlike the batch acetate study, cathodic pH behaved more as expected for five of the six batches, exhibiting a divergence of the anode and cathode pH as shown in Figure 17. The first two batches, the anode initial pH was not adjusted to pH and was instead left at the naturally occurring pH of the system (8.12 and 8.36 respectively) but was corrected for the remainder of the batches. This

can also be tied to the increase in hydrogen recovery from the batch acetate studies, which combined with the pH data, indicate the prevailing hydrogen evolution reaction. The stability of the anode pH can be attributed to the alkalinity of swine wastewater. While this was not quantified during the study, swine wastewater typically has an alkalinity of around 3,000 mg/L as CaCO_3 contributing to a strong carbonate buffering system, which is reported as being more effective than the phosphorus buffer system used in the acetate studies [21], [37].

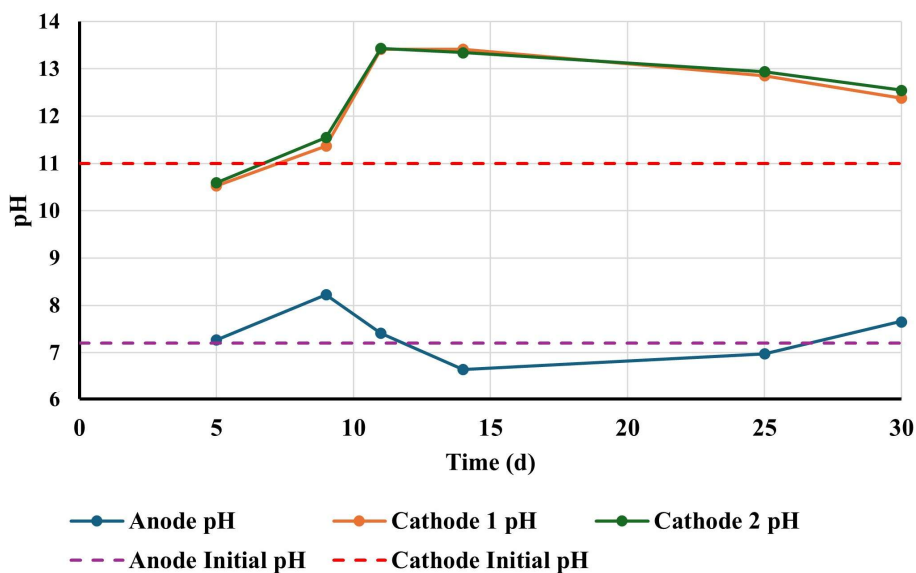


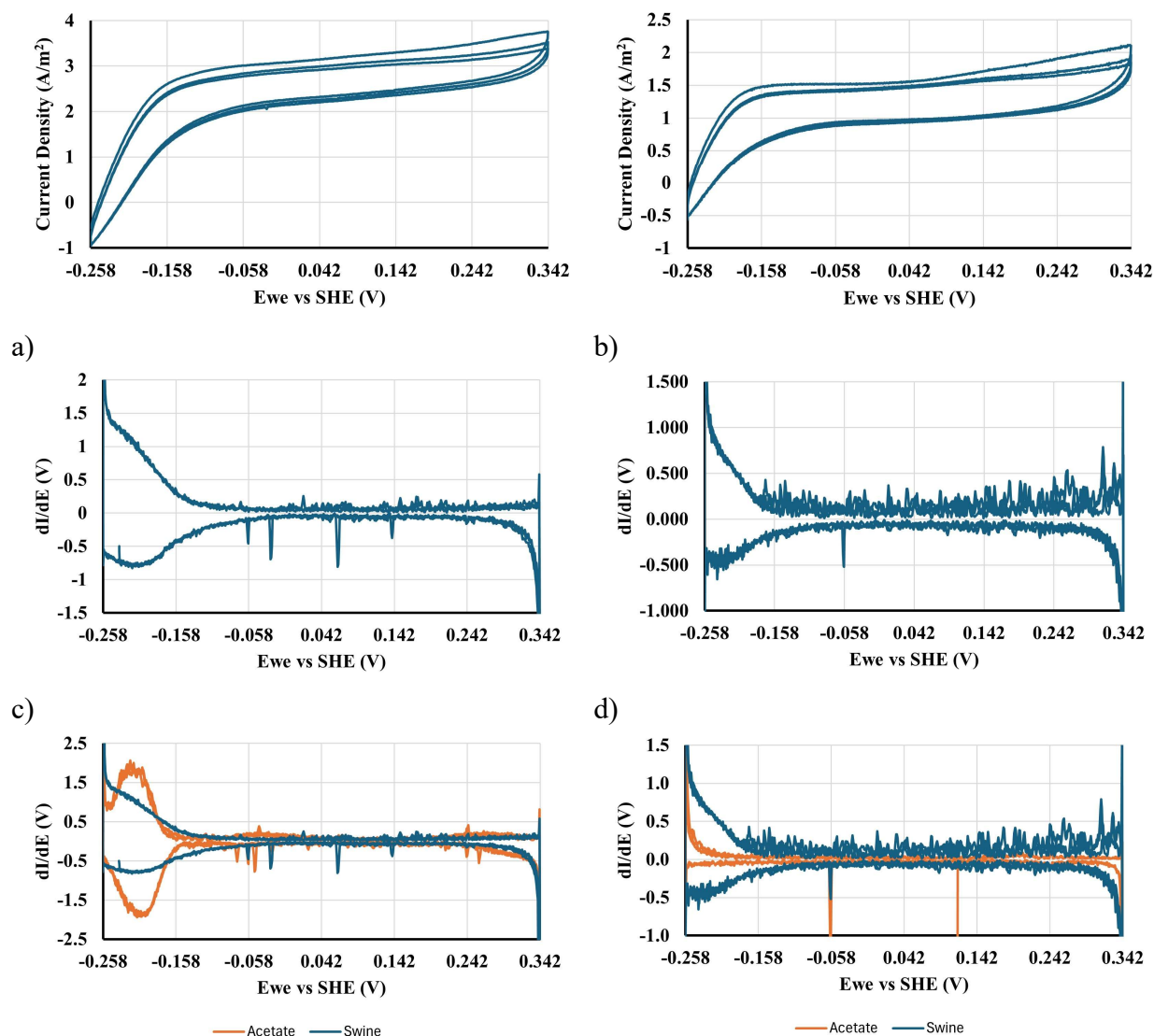
Figure 17: Variation in end of batch cycle pH

As there were no other studies found that assessed the performance of a substrate as complex as swine wastewater, this batch study establishes a unique set of results that achieve similar or better results than studies other complex substrates and real wastewaters. Despite the challenges posed by a substrate with a complex matrix and high variability in organic loading and solids (Figure 14), the system demonstrated adaptability and resilience, achieving high current densities and hydrogen production rates. Future considerations for this cell are increased catholyte monitoring and utilizing an alkaliphilic ARB such as *Geoalkilibacter* to reduce the pH gradient, in turn reducing the applied voltage required. Additionally, a controlled COD study should be completed

to determine the optimal range of CODs to produce high current density and subsequent hydrogen production.

3.3.2 Cyclic Voltammetry Scans

The cyclic voltammetry scans after the conclusion of the swine wastewater-fed batch cycles also exhibited significant hysteresis, disguising any active centers that may be present in Figure 18a and Figure 18b. This hysteresis was likely caused by the presence of high solids in the reactor that likely retained some of the potential applied to the cell during the scans despite scanning potentials that are amenable to ARB metabolism mechanisms. Due to the nature of the swine waste-fed system, there was also substrate present during the non-turnover CV; however, this was initially considered negligible due to the low current densities that indicated a relatively inactive biofilm, which may have contributed to the undesirable behavior in the non-turnover scan. These procedural challenges highlight the significance of the DCV plot in the identification of active centers. While neither CV plot shows a clear active center, the DCV are able to greater identify areas where there is heightened activity. Both the turnover and non-turnover DCV plots show heightened activity at the lower end of the scan, but neither have both a maximum and minimum activity within the scan range. The turnover DCV exhibits a local minimum (E_{red}) at -0.218 V vs SHE but does not have a local maximum. There appears to be the local maximum that exceeds the range of the plot. Based on the qualitative geometry of the curve, it appears that the E_{ka} for the turnover DCV will likely occur between -0.22 V and -0.24 V vs SHE. The non-turnover DCV plot also shows similar geometry to local minimums and maximums that exceed the lower range of the scan indicating that the E_{ka} is less than -0.258 V vs SHE.



e) f)
Figure 18: a) Swine turnover cyclic voltammetry scan, b) Swine non-turnover cyclic voltammetry scan, c) Swine turnover derivative cyclic voltammetry scan, d) Swine non-turnover derivative cyclic voltammetry scan, e) Acetate and swine derivative turnover cyclic voltammetry scans, f) Acetate and swine derivative turnover cyclic voltammetry scans

The results of the swine wastewater-fed are somewhat inconclusive due to the hysteresis and scan rate that did not capture all active centers; however, there does appear to be a shift in the active

center towards a more negative active center than the acetate fed system based on the partial activity peaks in the DCV as shown in Figure 18e and Figure 18f. In Chapter 2, the acetate-fed system had active centers primarily around -0.200 V vs SHE whereas the swine waste-fed MEC appears to have an active center below -0.22 V vs SHE after 30 days of acclimation. This is a relatively small shift in active center but could suggest the development of other strains or species of ARB. Regardless of the microbes present, the MEC was able to rapidly adjust to the new, more complex substrate and produce promising results that warrant further study. Future CV analysis of swine wastewater-fed MECs should identify more reliable methods of scanning to reduce hysteresis from solids, especially during turnover CVs that would require high substrate concentrations that typically also have high solids. Additionally, scans should include more negative potentials to capture accurate active centers for the microbes. For greater detail of the microbes that facilitate this conversion, DNA analysis should be completed to have a better understanding of the microbial metabolisms and genetic profiles.

3.4 Conclusions

In these studies, the novel flat-plate microbial electrolysis cell demonstrated reliability and promising results as the anode rapidly formed a robust ARB biofilm that was able to produce current densities and hydrogen performance consistent with or exceeding those reported in literature. While the current densities and hydrogen performance in the acetate batch cycles fell in the middle and lower part of the reported range with maximum values of 8.99 A/m² at 1.32 V and 0.99 L/L_{cathode}/d, respectively, these batches prepared the later studies to achieve higher results. As expected, the applied potential generally followed the change in current density ranging from approximately 0.7 to 1.3 V and produced high hydrogen purity in the cathode headspace with an average composition of 97±6% v/v. Cathodic recoveries (CCR or r_{cat}) varied greatly as well along

with coulombic recovery ranging from 18% to 78% (average of 47%) and cathodic hydrogen recovery ranging from 9% to 87% (average of 38%).

The subsequent continuously fed acetate operation improved these values as the biofilm continued to mature, with high substrate removal efficiencies averaging $98\pm 2\%$ during the 32 days of operation. These removals led to a more consistent current density output that averaged 7.31 A/m^2 at 1.18 V with a correspondingly high average coulombic recovery of 94% (coulombic efficiency of 97%). This also yielded improved hydrogen performance with a maximum volumetric hydrogen production rate of $7.29 \text{ L/L}_{\text{cathode}}/\text{d}$, hydrogen yield of $2.56 \text{ mol H}_2/\text{mol COD}$, and cathodic hydrogen recovery of 58.4% (averages of $4.98 \text{ L/L}_{\text{cathode}}/\text{d}$, $1.97 \text{ mol H}_2/\text{mol COD}$, and 49.8%, respectively) at an average purity of $100\pm 2\% \text{ v/v}$.

The batch swine-fed MEC study, also demonstrated promising data despite highly variable initial batch tCOD and TS with a maximum peak current density of 10.3 A/m^2 at 1.08 V. Like the acetate studies, applied voltage followed the current density and ranged from 0.8 to 1.4 V. Although not as high as the continuous acetate study, the gas performance data for the swine-fed operation fell within the expected range for complex substrate systems with a maximum volumetric production rate of $3.56 \text{ L/L}_{\text{cathode}}/\text{d}$, hydrogen yield of $0.83 \text{ mol H}_2/\text{mol COD}$, and cathodic hydrogen recovery of 46.2% (averages of $1.87 \text{ L/L}_{\text{cathode}}/\text{d}$, $0.54 \text{ mol H}_2/\text{mol COD}$, and 30.2%, respectively) at an average purity of $95\pm 2\% \text{ v/v}$.

The cyclic voltammetry analysis exhibited significant hysteresis at higher potentials making identification of active centers difficult; however, by use of derivative CV plots, denoising led to identification of a consistent active center at -0.200 V vs SHE after acclimation to acetate and provided indication of an active center outside of the scan range between -0.22 and -0.24 V vs SHE for the swine-waste acclimated biofilm. Although there was a slight shift to lower potential,

this appears to indicate consistency across previously identified signatures (half saturation potential, or E_{KA}) for dominant Anode Respiring Bacteria (ARB), mainly *Geobacter* spp.

These findings demonstrate the resiliency of the MEC platform under diverse conditions as well as the viability for swine wastewater as a substrate for the novel flat-plate microbial electrolysis cell for realistic scale-up. The robust biofilm formation, high current densities, and gas performance indicate a strong potential for scaling and application in sustainable hydrogen production from swine wastewater.

3.5 Future Work

Before scaling up this platform to accommodate higher flow rates and real-world treatment volumes, multiple areas for refinement were identified through the studies described above. Addressing these limitations through targeted experiments will be critical for maximizing performance, reducing energy demands, and enhancing the long-term viability of the novel flat-plate MEC platform. Key areas of focus should include efforts to determine the system's true maximum current density, minimizing internal and external losses, and improving operability.

First, as mentioned in previous sections, the novel flat-plate MEC achieved near complete acetate removal during continuous flow operation, indicating efficient substrate utilization under those conditions. However, it is notable that the swine wastewater substrate, despite its complexity and variability, achieved higher peak current densities than either batch or continuous acetate feeding. This suggests that the system is capable of handling more concentrated substrates and that its current output may be limited by acetate availability or retention time in the tested configurations. Therefore, increasing the influent acetate concentration or decreasing the hydraulic retention time in future trials may provide the conditions necessary to reach the true maximum current density potential of this platform.

Second, improving the conversion efficiency of current to hydrogen is something that must be addressed. Observed losses in electron recovery at the cathode were likely driven in large part by poor pH regulation in the cathodic chamber, which can significantly inhibit hydrogen evolution. Implementing a buffering system or other mechanism to stabilize cathodic pH would mitigate these losses and improve hydrogen yields. In addition, exploring cathode materials that are highly selective for the hydrogen evolution reaction, such as nickel-based alloys or other catalytic surfaces, could further reduce overpotential and energy consumption while improving gas production efficiency.

Third, while the swine wastewater substrate demonstrated promising performance, it also exhibited substantial variability in initial total COD and solids content. More targeted studies are needed to better understand the specific ranges of COD and TS that optimize microbial activity and current production. Identifying these operational windows will be essential for applying this technology to real-world waste streams with fluctuating composition.

Overpotential losses related to pH gradients between the anode and cathode represent another area with considerable room for improvement. The anode biofilm, assumed to be primarily composed of *Geobacter*, requires a neutral pH environment to maintain metabolic activity. However, large pH gradients can reduce overall system efficiency. Future work should explore inoculating the MEC with an alkaliphilic microbial enrichment predominated by known alkaliphilic ARB such as *Geoalkilibacter* species, which can tolerate and even thrive under elevated anode pH conditions. This could help raise the anolyte pH and reduce the pH gradient from 7.2-11 to a 9-11 range, reducing the energy required to operate the MEC.

Finally, to address a number of practical and performance-based limitations observed during early trials, a second-generation flat-plate MEC reactor was fabricated with multiple design

enhancements. These include repositioning the reference electrode to the side of the reactor to allow for better control of liquid level and headspace volume, decreasing the distance between anode and cathode to reduce internal resistance and applied voltage requirements, adding influent and effluent ports to the cathode chamber for improved gas-liquid management and easier pH sampling, enlarging the gas collection outlet to allow for better compatibility with gas bag fittings and minimize leakage, and incorporating nickel or stainless steel–nickel alloy cathodes to improve HER selectivity and reduce the system’s overall energy input. Another aspect to be studied in the long term is to connect multiple flat plate MECs in series or parallel to achieve scalability with superior conversion of wastewater COD to hydrogen gas. Together, these improvements are expected to increase data quality, improve scalability, and better position the platform for pilot-scale deployment.

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