Pilot study of operational parameters in microbial electrolysis cell with dark fermentation

By

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Abstract

Biohydrogen production via dark fermentation (DF) and microbial electrolysis cells (MEC) demonstrate great potential for sustainable hydrogen generation. In this study, DF and MEC were coupled as a hybrid reactor and evaluated using brewery wastewater. The effect of hydraulic retention time (HRT) and buffer concentration on reactor performance were assessed by current density and hydrogen production rate (HPR). Current density and HPR were highest at 48h HRT as compared to 60h, 36h, and 24h HRTs. When adjusting to 73mM from 146mM phosphate buffer solution, current density and HPR decreased by 22% and 28%, respectively. These results suggest 48 h HRT and 146 mM buffer concentration can be used to improve this reactor's performance. Future experiments should be replicated over longer periods to ensure microbial communities are adjusted and the internal environment is stabilized.

Keywords: Dark fermentation; microbial electrolysis cell; wastewater; biohydrogen

1. Introduction

As human populations have continued to grow and energy demands increased, our consumption of fossil fuels has reached unsustainable levels. Alternative energy, which includes renewables and low carbon-emitting sources, must be investigated as a means for long-lasting human development. Hydrogen is a promising energy alternative that produces zero carbon emissions during combustion and has a high energy content of 120 kJ/g [1]. Although hydrogen combustion results in cleaner emissions, hydrogen production often requires fossil fuels; in fact, 96% of hydrogen gas (H_2) production in 2012 was from fossil fuels [2,3]. Hydrogen gas must be produced from sustainable, renewable resources at low costs if it is to be competitive with hydrogen production by natural gas and oil consumption [4].

Biohydrogen production via dark fermentation (DF) has been investigated for its dual functionality in wastewater treatment and hydrogen production. However, DF has low hydrogen conversion efficiencies of 18-38% [5]. Coupling DF processes with microbial electrolysis cells (MECs) in a hybrid, DF-MEC may optimize biohydrogen recovery. MECs require a low supplied voltage, which promotes the thermodynamically unfavorable microbial oxidation of substrates, such as volatile fatty acids (VFAs), to generate H_2 [6,7]. MECs alone have been able to achieve hydrogen conversion efficiencies ranging from 72-93% [5]. Implementing DF within a single-chamber MEC would allow VFAs produced from fermentation processes to be consumed by anodic microbial communities. Li et al. previously demonstrated a hydrogen production rate (HPR) of 3.43 $m^3m^{-3}d^{-1}$ through this process in a hybrid reactor using corn-stalk [8]. However, the study only tested small (64 ml) reactors in a two-stage process, which may limit the system's scalability.

The current study aims to test a large-scale (10 L), hybrid DF-MEC with wastewater substrate from brewery effluent. Beer brewing is estimated to produce 10 liters of wastewater per liter of beer, which is why it was chosen as the influent for this study [9]. Marone et al. demonstrated that using agricultural wastewater in a DF-MEC, two-stage system increased HPR by 13 times more than that of only DF [4]. Given this success with agro-industrial wastewaters in a two-stage DF-MEC, it is important to investigate the performance of a hybrid reactor with the goal of reducing operational costs associated with two-stage designs. Additionally, previous studies have only investigated the performance of agro-industrial wastewater, and brewery wastewater in MECs has yet to be investigated.

This study incorporated the use of actual brewery wastewater in a novel, hybrid DF-MEC reactor. DF-MEC performance was evaluated for two outputs: HPR and current density. In MECs, current density (A/ m^2) describes the amount of current flowing through the anode surface area. MECs require a supplied voltage, therefore current density is a significant measure of energy efficiency. Furthermore, higher current densities have been linked with higher hydrogen yields [10]. HPR is the desired output for most MEC operation and thus was considered an important parameter for this study.

Each performance parameter (HPR and current density) was studied under various hydraulic retention times (HRTs) and buffer concentrations. Gil-Carrera et al. demonstrated that in a double-chamber MEC, longer (10 h and 7 h) HRTs were correlated to lower energy consumption than short (4 h) HRTs [11]. Previous studies suggested that higher buffer concentrations improve MEC performance because it can reduce internal resistance and further increases substrate conductivity [5,12]. However, it is necessary to optimize HRT and buffer concentration such that operational costs are minimized and biologically produced H_2 can be

generated at a pilot-scale. Very few MEC studies include the use of actual wastewater as an input, and therefore are missing the link between wastewater characteristics and MEC performance parameters. In summary, the objectives of this research are to:

- Investigate the effect of HRT on DF-MEC performance outputs, defined by current density and HPR.
- Determine the effect of buffer concentration on MEC performance outputs.

2. Materials and Methods

2.1 Reactor design

The DF-MEC reactor used in this study was previously constructed by Andrew G. Miller and Dr. Lakhveer Singh [13]. The reactor was a 10 L, single-chamber tank constructed from acrylic plastic with 5 electrode pairs, each 100 cm long. Electrodes were made from carbon cloth (E-Tek, USA) and fastened to stainless steel frames (0.45 mm thickness) with nylon screws. There were 5 cathodes, each with a total surface area of 563 cm^2. Anodes consisted of a total of 18 pieces of carbon cloth, divided into groups of three and fastened by a titanium frame (Grade 2, 0.5 mm thickness), with each piece being 563 cm^2. The anode biofilm used in this study was transferred from other MECs using acetate and glucose substrates, which were enriched with local domestic wastewater [14]. The cathode was treated with a Molybdenum Phosphide (MoP) catalyst to promote hydrogen generation [15].

In the same tank as the MEC electrodes, fermentation beads were added in batch to increase available pore space for microbial communities. These were acrylic latex silicone beads, which were made according to Wu et al. [16]. There were inlets and outlets at the top and bottom of the tank, and through the fermentation beads, for recirculation pathways. The entire reactor was covered in aluminum foil to eliminate light exposure.

2.2 Brewery Wastewater

Samples of brewery wastewater were collected from Mazama Brewing in Corvallis, Oregon. Wastewater was collected after the final discharge stage of the brewing process. Samples were stored in refrigerators at approximately 1.6 degrees Celsius for up to one week, or until time of use, to avoid biodegradation. Prior to HRT and buffer concentration experiments, the brewery wastewater was characterized by chemical oxygen demand (COD) and high-performance liquid chromatography (HPLC) tests. The COD of brewery wastewater influent was measured using standard methods from United States Environmental Protection agency, Method 410.4 (1993) [18]. COD was used only to ensure that wastewater was consistently diluted between HRT and buffer concentration trials. Wastewater used to study the effect of HRT was diluted to 10 g COD/L. Wastewater used to study the effects of buffer concentration was diluted to 5 g COD/L and ran at 24 h HRT. There was no pretreatment prior to testing in the DF-MEC and initial conditions of wastewater are recorded in Table 1 of this text's *Results and Discussion* section.

2.3 Operation

Throughout all experiments, the temperature of the reactor was controlled at 37 degrees Celsius using 3 L of recirculating fluid. Additionally, pH was controlled with 100 mM sodium hydroxide buffer solutions to achieve consistent pH between 6.6-7.0. A peristaltic pump (MasterFlex 7523-40, Barnant, CO) controlled influent solution through the thermal heating tank and into the MEC. The applied voltage between cathodes was regulated using a data-acquisition system across 0.1 Ω resistors (2700, Keithly, MA). For all treatments, influent was amended with 0.02% (volume/volume) chloroform ($CHCl_3$) to suppress H_2-consumption [13]. Nutrient and

mineral solutions, KCl and NH_4Cl were added to wastewater to support cell metabolism, as previously described [17].

For the first set of experiments, the effect of HRT on reactor performance was studied using wastewater was mixed with 73 mM phosphate buffer. The 73 mM phosphate buffer was prepared to 1 L of tap water with 4.3g $NaH_2PO_4H_2O$ and 11.3g $Na_2HPO_4 \cdot 7H_2O$. The brewery wastewater was diluted to 10 g COD/L and loaded into the influent tank after being mixed with the 1 L of 73 mM phosphate buffer. HRT was first set to 60h HRT, which ran for a total of 4.5 days. After this time the flow rate was adjusted to achieve 48h HRT for approximately 3.75 days. Again, the pump was adjusted for 36 h and 24 h HRT, each which ran for 4.75 and 4 days, respectively.

The effects of buffer concentration were examined by consistently operating the reactor under 24 h HRT. Again, 73mM phosphate buffer was prepared as described above, in addition to 146mM (8.6g $NaH_2PO_4H_2O$ and 22.6g $Na_2HPO_4 \cdot 7H_2O$). First, 146 mM phosphate buffer solution was mixed with the brewery wastewater and mixed before pumping. This solution ran continuously for two days before switching directly to wastewater mixed with 1 L of 73 mM buffer solution, which also ran for approximately two days.

2.4 Measurements and Calculations

HPLC analysis was performed to determine the concentration of VFAs present in the reactor. First, liquid effluent samples were collected from three different test valves on the reactor: the bottom, middle, and top of the MEC. Samples were placed into 1 ml centrifuge tubes and centrifuged for five minutes, or until solids coagulated. The samples were placed in the

chromatograph (Agilent Technologies 1200 Series, Santa Clara, CA) for analysis following methods previously described [8].

Current density and hydrogen percentage were measured throughout both the HRT and buffer concentration experiments. Current was continuously recorded throughout operation and then was divided by the total cathode surface area (563 cm^2) to yield current density. Total biogas volume from the MEC was measured continuously using a gas meter (Ritter, TG 0.5 Plastic). The biogas was then analyzed using a gas chromatograph (SRI Instruments, Torrance, CA; Shimadzu, Japan), which directly gave the hydrogen percentage as a percentage of total biogas volume [19]. Then, HPR was determined by multiplying hydrogen percentage by the total biogas volume and normalizing over the 10 L working volume of the reactor.

3. Results and Discussion

Brewery Wastewater characterization

Prior to loading the DF-MEC with brewery wastewater for HRT and buffer concentration experiments, COD and HPLC analyses were conducted. Table 1 shows characteristics of the wastewater just before being amended with phosphate buffer, chloroform, and being diluted to 10 g COD/L. The wastewater used in the experiments was from the same batch, and therefore it was assumed that similar ranges in parameters remained. This characterization is included to demonstrate that the wastewater had no VFAs detected prior to dark fermentation.

Table 1. Brewery wastewater characteristics prior to DF-MEC treatment for HRT experiments, which was diluted to 10 g COD/L.

Parameter	Range
Chemical oxygen demand (mg/L)	20,000-60,000
pH	4.0-5.0
VFAs: Lactic, Formic, Acetic, Propionic acids (mg/L)	Not detected
Alcohols (mg/L)	Not detected
Total carbohydrate (mg/L)	18,000-55,000
Conductivity (mS/cm)	0.7-0.8
Temperature (ºC)	37

Effect of Hydraulic Retention Time

Wastewater was sampled three times for HPLC during each treatment at the center of the reactor. Table 2 shows averaged results of VFA concentration by type, as well as the total amounts for each HRT treatment. The total glucose content for all HRT influents was not detectable, suggesting that glucose was consumed by the DF mechanisms in the reactor. The total VFA content for 24h HRT was 4264 mg/L. Brewery wastewater in the reactor during the 36 h HRT treatment had a total of 5857 mg VFA/L. During 48 h HRT, VFA content was 4659 mg/L, and VFAs after the 60 h HRT treatment were significantly less, at 1280 mg/l.

The VFA content is an indicator of the metabolic productivity of microbes during DF to convert glucose to acids, as well as an indicator of the rate of MEC bacteria to oxidize those acids. Therefore, Table 2 suggests that during 60h HRT there VFA production was much less from DF *or* a higher uptake of VFAs by the MEC. Total VFA may be lower in the 60h HRT treatment because it is enough time for DF to release VFAs and for MEC microbes to metabolize the acids.

Table 2. HPLC data for brewery wastewater characterization (influent 10 g COD/L) used in HRT experiments. Concentrations are averaged from three samples. No detection is indicated by ND.

HRT (hr)	Glucose (mg/l)	Lactic acid (mg/l)	Formic acid (mg/l)	Acetic acid (mg/l)	Propionic acid (mg/l)	Butyrate (mg/l)	Ethanol (mg/l)	Total (mg/l)
24	ND	ND	461	1097	1614	ND	1092	**4264**
36	ND	ND	750	1619	1822	79	1580	**5857**
48	ND	ND	489	1091	2255	105	720	**4659**
60	ND	53	119	136	472	418	427	**1280**

HRT was investigated because it is correlated to substrate loading rate in the MEC and contributes to mixing. Li et al. (2014) demonstrated that overloading MECs with substrate negatively impacted current density and inhibited exoelectrogen activity [8]. Furthermore, HRT is related to flow rate, which was the only mixing mechanism in the DF-MEC reactor. Long HRTs run the risk of starving the system of glucose and not mixing sufficiently, thus reducing mass transfer of nutrients. Conversely, short HRTs may overload the MEC with substrate and even require a flow rate capable of displacing microbial communities.

Current density was studied as an important performance parameter because the driving mechanism of transferring electrons onto the anode surface is due to the microbial uptake of VFAs. Figure 2 shows the current density (A/m^2) for 60, 48, 36, and 24 h HRT. The average current density for 60 h HRT under these conditions was 10.9 A/m^2, which ran over the course of four days. Current density for 60 h HRT demonstrated the most variance of all HRT treatments, with a standard deviation from the mean of 1.2. Current density is related to the amount of hydrogen being produced by microbial oxidation; however, it does not necessarily correlate to how much hydrogen is recovered by the system. Therefore, current density is only representative of the productivity at the anode. As seen in Figures 3 and 4, hydrogen percentage and HPR during 60 h HRT conditions averaged 12.1% and 2.35 L H_2 L^{-1} d^{-1}, respectively.

Current density during 48 h treatment averaged 12.1 A/m^2 and had a standard deviation of 0.7. In addition to higher current densities as compared to 60 h HRT, the 48h trial had a hydrogen percentage and HPR of 17% and 3.29 L H_2 L^{-1} d^{-1}, respectively. The reduction in HPR during 60 h HRT as compared to 48 h HRT may be attributed to the slower substrate loading rate. Longer HRTs may cause a lack of available substrate to hydrogen-producing bacteria, whereas slightly shorter HRT (48 h) may meet both the loading and mixing needs of the bacteria. Furthermore, the VFA detected during 48 h HRT was higher than 60 h treatment, which may indicate that both DF and the microbial electrolysis processes were most efficient under this condition.

During 36h and 24h treatments, current densities and HPRs were much lower than either 48 h and 60 h treatments. The current density during 36 h HRT was 7.4 A/m^2 and a standard deviation of 0.5— significantly (58%) less variation than 48 h HRT. Furthermore, the 24 h HRT had a current density of 5.0 A/m2 and a standard deviation of 0.5. In addition to lower current densities, 36 h and 24 h HRTs had lower hydrogen percentages (8.8% and 6.1%, respectively). The HPRs of 36 h and 24 h HRT were 1.7 L H_2 L^{-1} d^{-1} and 1.2 L H_2 L^{-1} d^{-1}. Although performance was lower, the shorter HRTs (36h and 24h) had similar VFA contents to 48 h HRT: 4264 mg/l, 5857 mg/l and 4659 mg/l, respectively (Table 2). This suggests that even though the DF process may have been actively generating available substrate (VFAs) for the MEC, the exoelectrogens did not have enough time to oxidize the substrates (i.e., transfer electrons and generate a current).

These results suggest that VFA production from DF and microbial uptake of VFAs does vary with HRT. HRT must be long enough to allow sufficient substrate exposure and to not displace bacteria, but also short enough to ensure thorough mixing and avoid overconcentrating

microbial communities. Based on these experiments 48 h HRT proved to be optimal in terms of hydrogen production, as demonstrated by achieving the highest current density and HPR of all treatments.

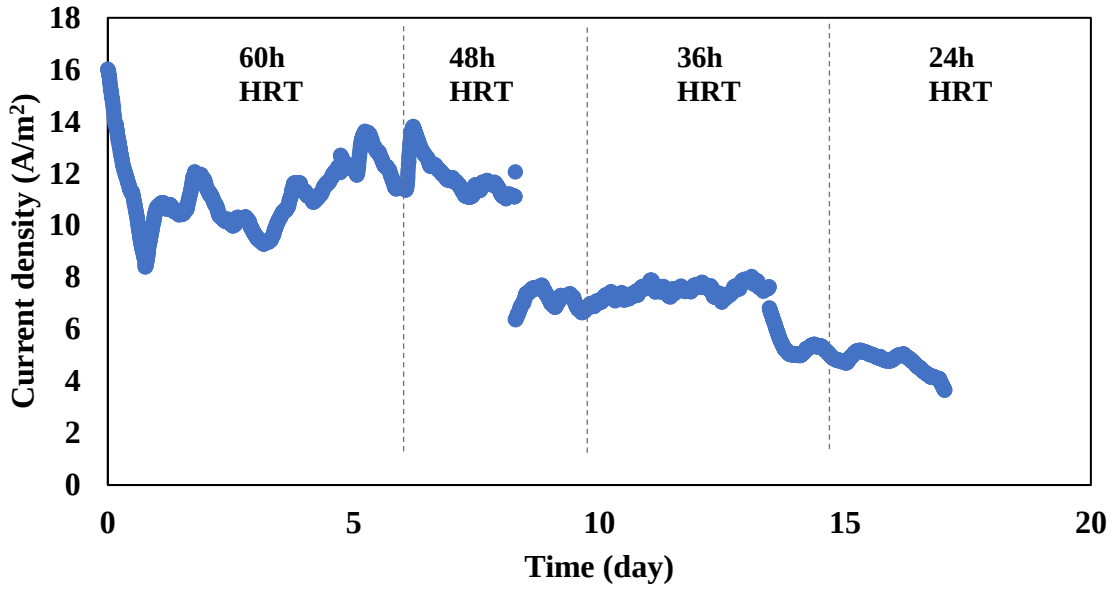

Figure 2. Current density at HRT of 60, 48, 36 and 24 hr. Current density was calculated by dividing the total current reading by the cathode surface area. The brewery wastewater for these experiments was initially 10 g COD/L.

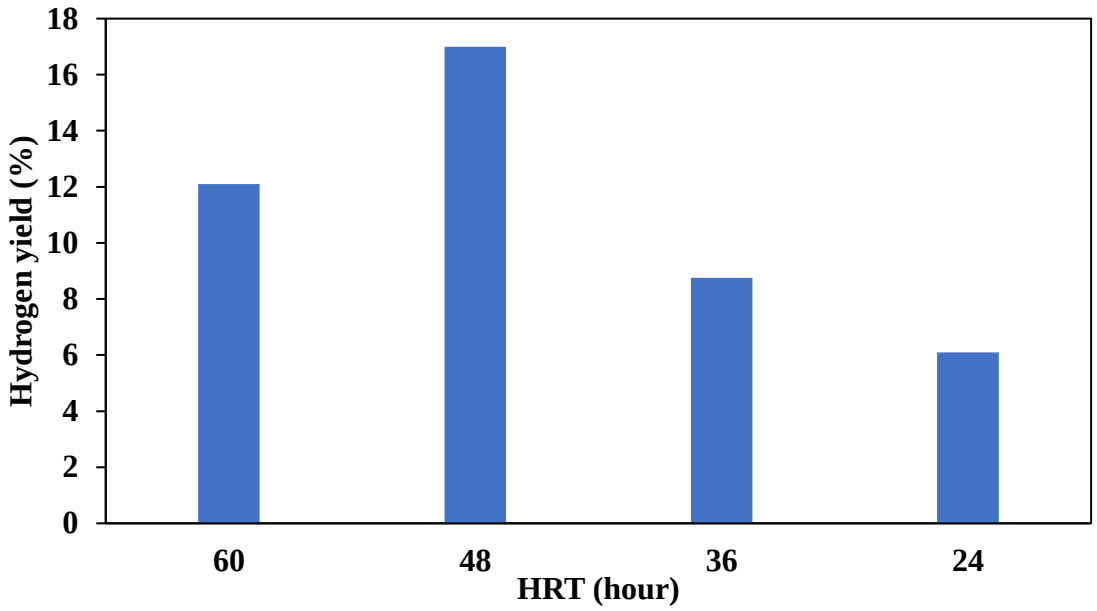

Figure 3. Percent of hydrogen yield out of total biogas production from 10 g COD/L brewery wastewater. Error is not displayed because only one trial was taken under each condition.

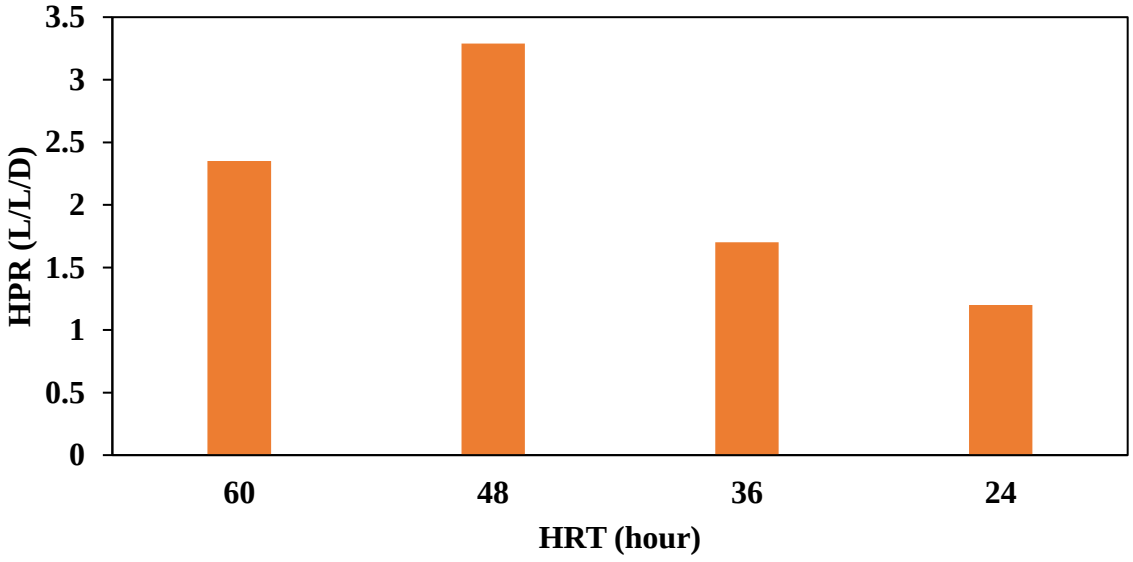

Figure 4. Hydrogen production rate (HPR) for 60, 48, 36, and 24 h HRT with 10 g COD/L influent brewery wastewater. Error is not displayed because only one trial was taken under each condition.

Effect of Buffer Concentration

Higher buffer concentrations have been shown to improve MEC performance, but often at a decreasing rate of improvement [5, 12]. This trend has implications for cost when considering scaling MEC technology. Additionally, buffer concentration is particularly important to study with regards to actual wastewater since wastewater often has variable substrates, and buffers may control for this range and reduce internal resistance.

Table 3 displays results of HPLC testing for the brewery wastewater used in the buffer concentration experiments prior to being amended with the phosphate buffer treatments. The average VFA content was 1546 mg/l, which was operating under a 24 h HRT throughout the four-day trial. HPLC data was only taken at one point in the experiment (just before the 146 mM buffer treatment), therefore limiting full comparative analysis between 146 mM and 73 mM treatments.

Table 1. HPLC data for brewery wastewater characteristics used in buffer concentration experiments (5 g COD/L). ND indicated concentrations were not detectable.

Sample location	Glucose (mg/l)	Lactic acid (mg/l)	Formic acid (mg/l)	Acetic acid (mg/l)	Propionic acid (mg/l)	Butyrate (mg/l)	Ethanol (mg/l)	Total (mg/l)
Bottom	60	85	366	356	143	117	149	**1276**
Middle	185	ND	377	364	549	72	255	**1802**
Top	84	ND	314	321	486	70	271	**1546**
Average	110	28	352	347	393	86	225	**1541**

Figure 5 shows the effect of buffer concentration on current density on the continuously running reactor. The effect of reducing buffer concentration from 146 mM to 73 mM caused the average current density to decrease 23%; from 12.9 A/m^2 to 9.9A/m^2, respectively. While the DF-MEC reactor was loaded with 73 mM buffer concentration, current densities had a standard

deviation of 1.25. During operation with 146 mM buffer concentration, current density had a standard variation of 0.67. The reduced variance of current density with higher buffer concentration may be attributed to the chemical mechanisms of phosphate buffer to stabilize the microbial environment by reducing the internal resistance of the MEC.

Table 4 summarizes the aforementioned effects of buffer concentration on current density, as well as the percent of hydrogen present in biogas and HPR. Figures 6 and 7 show that hydrogen percentage and HPR were higher while loading influent with 146 mM buffer solution as compared to 73 mM solution. This set of experiments demonstrated that decreasing buffer concentration by half did not directly result in directly 50% of performance outputs (current density, hydrogen percentage and HPR). Despite decreasing buffer concentration from 146 mM to 73 mM, current density was only 23% lower and HPR was only 28% less.

Further experiments should be conducted to test multiple buffer concentrations such that the costs of resource (buffer) consumption are kept down and hydrogen percentage remains high. In addition to running only one trial, this study is further limited since both buffer treatments were only run for two days- which may not have been enough time for the system to stabilize. Future studies should include a controlled treatment, in which no phosphate buffer is added. Including a control is a necessary to determine whether reduced performance was a result of decreased buffer concentration or a function of time.

Table 2. Average current density for 146 mM and 73 mM buffer concentrations with 24 h HRT.

Buffer concentration	Average current density ($A/m^{2)}$	Hydrogen percentage of biogas (%)	HPR (L/L/D)
146 mM	12.9	25	2.5
73 mM	10.0	18	1.8
Percent difference	23%	28%	

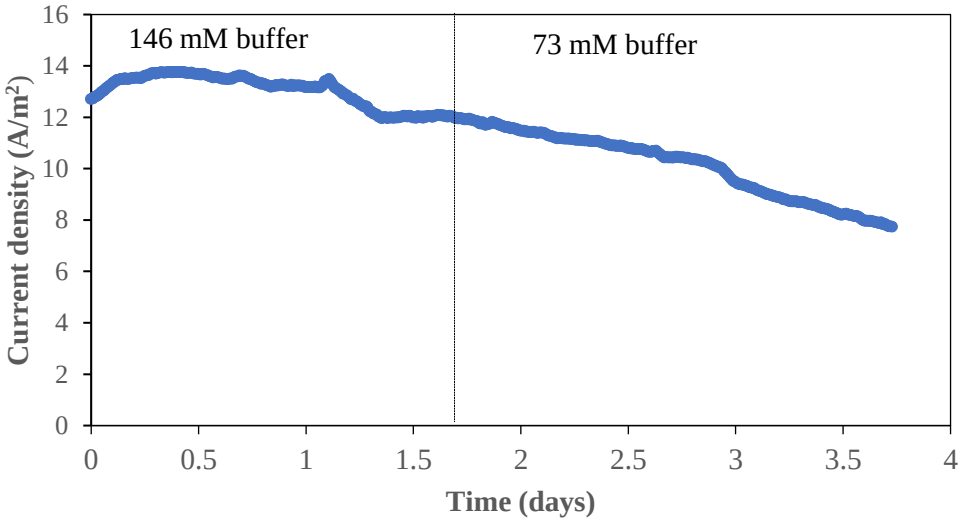

Figure 5. Effect of buffer concentration on current density during a four-day (89 hr.) trial. Brewery wastewater influent was 5 g COD/ and was operated under a 24 h HRT.

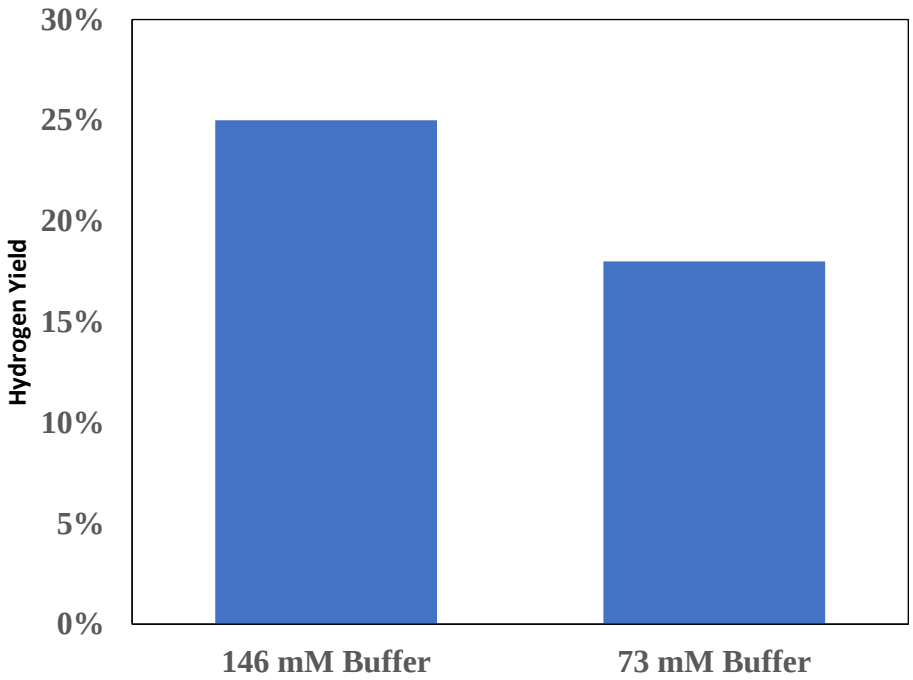

Figure 6. Hydrogen yield from Brewery wastewater (5g COD/l) based on total electrons at 146 mM and 73 mM Buffer concentration during 24 h HRT

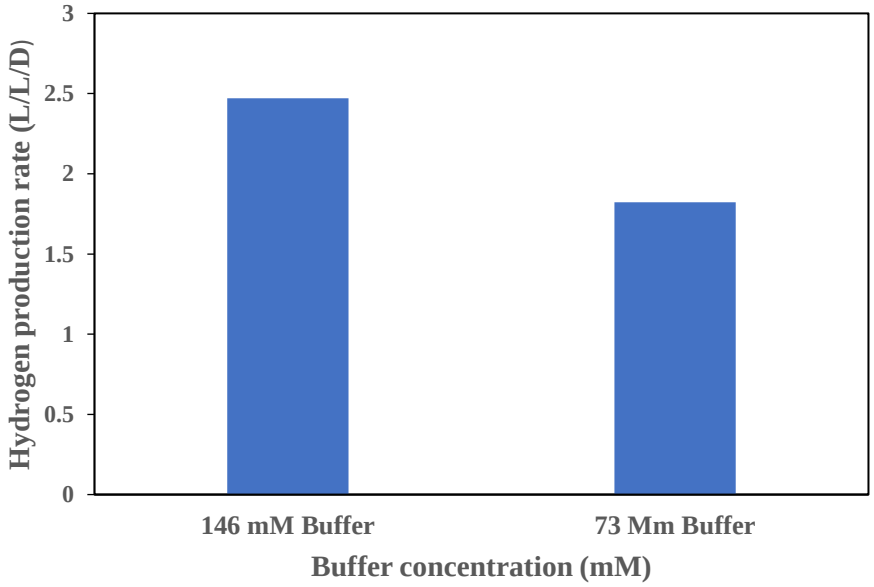

Figure 7. Hydrogen production rate from brewery wastewater (5g COD/l) based on total electrons for 146 mM and 73 mM buffer concentrations during 24 h HRT.

4. Conclusions

In this study, a hybrid DF-MEC reactor was evaluated on two performance outputs: current density and HPR. The effect of HRT on these outputs demonstrated that longer HRT improved performance from 24 h to 48 h HRT but peaked and did not improve performance at 60 h treatments. Current density and HPR were highest during 48 h HRT, suggesting an optimal rate may be 48 h. Further research is necessary to test HRTs over a longer period, at repeated trials, and for various treatments. Reducing the buffer concentration by 50% (from 146 mM to 73 mM) did not directly correlate to 50% lower performance, however did result in 22% lower current density and 28% lower HPR. This study lacked repeated trials of treatments and did not allow enough time for the DF-MEC reactor to adjust to the various parameters. Therefore, further research is necessary to assess the effect of HRT and buffer on this large-scale, hybrid reactor

Appendix

A. Supplementary MEC background

MECs primary mechanism is oxidation (electron transfer) via microbial metabolic processes. Exoelectrogens are bacteria capable of transferring electrons outside of their cell membrane to a solid acceptor outside of the cell using conductive filaments. These bacteria form a thin biofilm at the anode of the MEC [13]. The most common species of exoelectrogens used in MECs are *Geobacter sulfurreducens* and *Shewanella oneidensis* [20]. As oxidation occurs, a current is generated and electrons are joined with protons at the cathode to generate H_2. Although MECs require a small supplied voltage for hydrogen production, traditional water electrolysis often requires higher current inputs— approximately 0.7 V versus 1.23 V, respectively [19]. The supplied voltage allows the biofilm to continue metabolizing organic matter by creating a small amount of space between the bacteria and the anode surface [13].

Although MECs offer many benefits to pollution reduction, there are several limitations to their scalability. To make the oxidation process thermodynamically favorable, a supplied voltage is required. The applied voltage is likely to be generated from a non-renewable source, such as natural gas, thus increasing the net emissions of MECs. Guo et al. (2017) discuss how low electrode surface area to reactor volume ratios, large internal resistance, and poor mass transfer require higher supplied voltages, and thus higher operational costs of MECs [21]. Another cost that limits the scalability of MEC is the difficulty to operate only pure bacteria cultures. If not controlled with buffer solutions, hydrogenotrophic methanogens and homoacetogens may consume hydrogen and reduce yields [6].

MECs can be either double- or single-chambered depending on the presence of a proton-exchange membrane between electrodes. Double-chambered reactors have higher H_2 recovery

and purity, but also require higher applied voltages due to high internal resistance and pH difference between chambers. Single-chambered MECs address the limitations of a membrane and have lower capital costs, lower internal resistance, and lower supplied voltage requirements than double-chambered reactors [21]. Controlling for hydrogen-consuming bacteria is a more common limitation for single-chambered MECs and should be optimized to ensure highest efficiency.

B. Figures

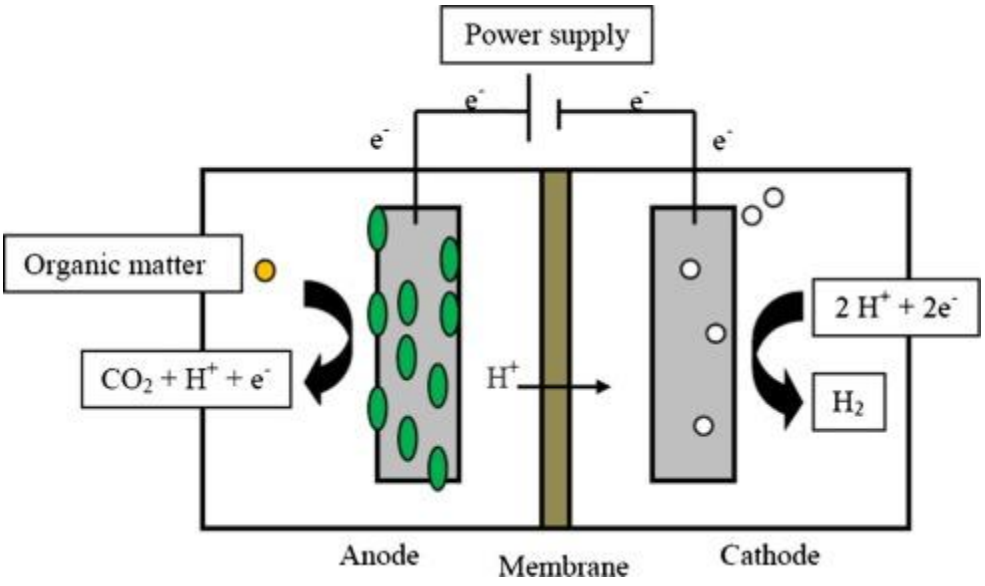

Figure 8. Schematic demonstrating the bioelectrochemical mechanisms of a double-chamber MEC, from Kadier et al. [3]. A single-chamber MEC has the cathode and anode in the same batch, without a proton exchange membrane.

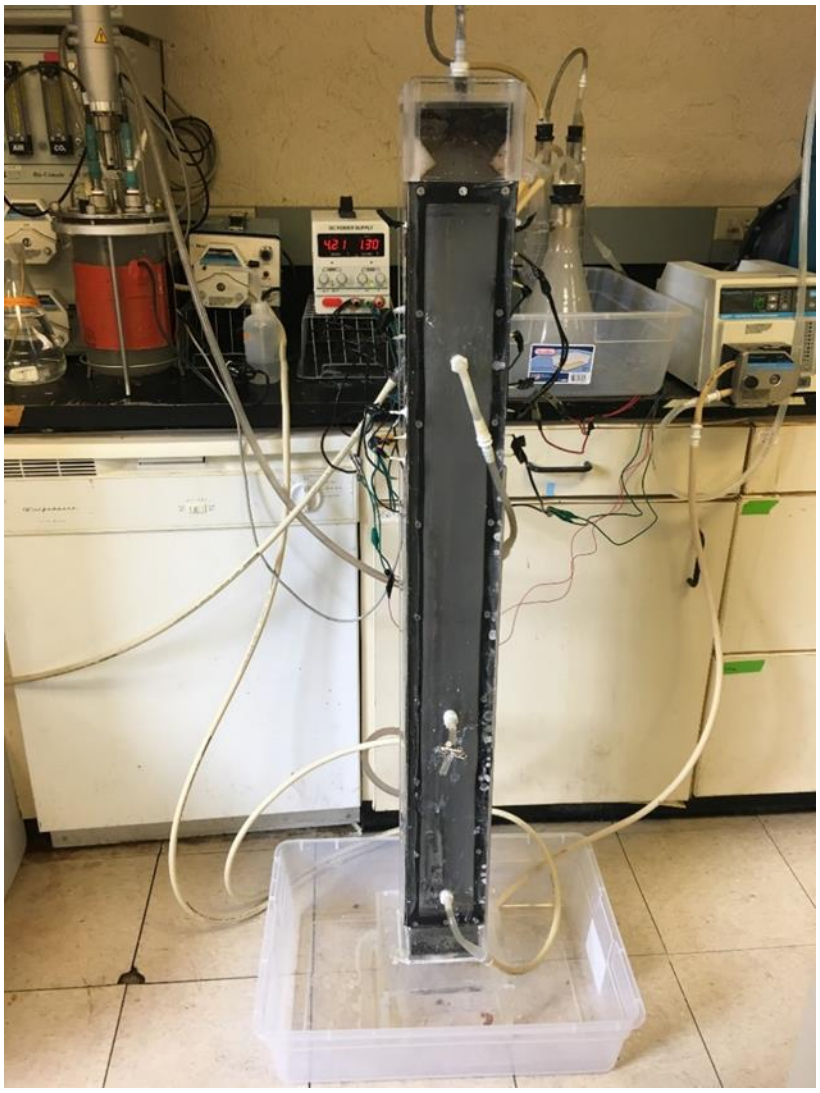

Figure 9. DF-MEC system connected to pH and thermal recirculation tank, power supply, gas collection chamber, and pump [13].

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