FINAL REPORT
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TREATMENT OF RENDERING WASTEWATER IN MICROBIAL FUEL CELLS WITH PEROXIDE PRODUCTION

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Lay Summary:

This project sought to develop and evaluate the applicability of microbial fuel cells (MFCs) for the secondary treatment of rendering wastewater. MFCs represent an upcoming wastewater treatment technology wherein the energy embedded in the chemical oxygen demand (COD) content of the wastewater is recovered as electrical energy or as value-added products. MFCs are potentially well-suited for high strength wastewater, such as rendering wastewater. Through the work conducted, we evaluated whether high rates and efficiencies of COD removal, concomitant with electrical current generation, can be achieved. An additional advantage of MFCs is the separation of ammonium (NH_4^+) ions produced from hydrolysis and fermentation of proteins, and subsequent recovery. Although this was a goal of the project, the rendering wastewater used in this project was low on protein concentrations, and thus the prospect of nitrogen recovery was not specifically evaluated. The electrical current produced in the MFCs can be used to produce hydrogen peroxide, an important chemical for rendering plants, which can be used on site for cleaning, disinfection, and/or odor control. Factors that affect the rate and efficiency of peroxide production were thus also evaluated. Finally, a prototype modular MFC was constructed to serve as a model for scaling up to a L-scale for a follow-up project. The future goals of work on MFCs for rendering wastewater treatment beyond those to be conducted through the next project would include exploring other co-products, as well as building a pilot-scale unit for conducting further studies.

Objective (s):

The overall objective of the proposed research was to develop MFCs for secondary treatment of rendering wastewater. The specific research objectives were:

1) Demonstrate COD removal from rendering wastewater with electrical current production in the anode of MFCs, following primary treatment.
2) Optimize the rendering wastewater HRT in the anode of MFCs to achieve high rates of NH_4^+ transport to the cathode. This objective had to be omitted since the rendering wastewater used in the project was found to have very low protein concentrations.
3) Determine the potential peroxide production in the cathode of MFCs fed with rendering wastewater at the anode through studying different cathode parameters that affect peroxide concentrations and production efficiencies.
4) Design a modular prototype system for rendering wastewater treatment that combines the optimum anode and cathode configurations determined through Objectives 1-3.

Project Overview:

Introduction

Rendering wastewater contains high levels of COD, which incurs significant aeration costs in secondary biological treatment, following primary treatment with DAF to remove the solids. The COD content in the wastewater represents a significant amount of chemical energy that could be potentially recovered to make rendering operations more sustainable and economic. Anaerobic technologies are preferred for energy-neutral or –positive secondary treatment, since

no aeration is required, and methane (CH_4) is produced. Microbial fuel cells (MFCs) represent an upcoming wastewater treatment technology, wherein anaerobic processes yield the generation of an electrical current rather than CH_4 gas. This is made possible by the activity of anode-respiring bacteria (ARB) that respire to electrodes while oxidizing anaerobic fermentation products. The electrical current then can be used at the cathode to produce a suite of products, including electrical power, hydrogen (H_2) gas, caustic soda, peroxide, among many others. MFCs could also potentially follow a membrane filtration step for primary treatment, as proposed and studied by Husson and co-workers at Clemson through previous ACREC funding.

MFCs have been studied extensively for domestic wastewater treatment over the past decade. Results in terms of treatment performance have been adequate, but energy recovery has been low. This is because the low strength of domestic wastewater. Since ARB grow on electrodes as biofilms, diffusional limitations make it so that high strength wastewater is needed to produce large amounts of electrical current, and thus high rates and extents of energy recovery. Thus, rendering wastewater could potentially be ideal for MFCs. Rendering wastewater does contain high concentrations of fats and proteins. There has been significant work done on anaerobic conversion of fats, oils and greases in anaerobic bioreactors, but none directly with MFCs. Nonetheless, since the microbial communities in anaerobic digesters for example are often like those in MFCs, we can expect good removal and conversion for the COD in rendering wastewater. The high protein content of rendering wastewater is often considered a problem for anaerobic treatment, because of the sensitivity of the methane-generating microorganisms to NH_4^+ produced from protein hydrolysis and fermentation, but ARB can sustain activity even at high NH_4^+ concentrations[13], which suggests favorable application for rendering wastewater.

The key advantage MFCs bring to rendering wastewater treatment is the ability to produce a range of products other than CH_4. In the most traditional form of an MFC, oxygen (O_2) is reduced at the cathode to produce electrical power. This requires the use of expensive metal catalysts however. More exciting is the recent discovery that peroxide can be produced at the cathode of an MFC (Figure 1), while using simple and cheap carbon electrodes for O_2 reduction. In addition, the potential difference between the anode and the cathode results in the movement of cations from the anode to the cathode, resulting in separation and removal of NH_4^+ ions from the wastewater. At the cathode, peroxide production results in an increase in the pH, allowing to volatilize and recover N as NH_3 gas, which could be further converted to fertilizer co-product.

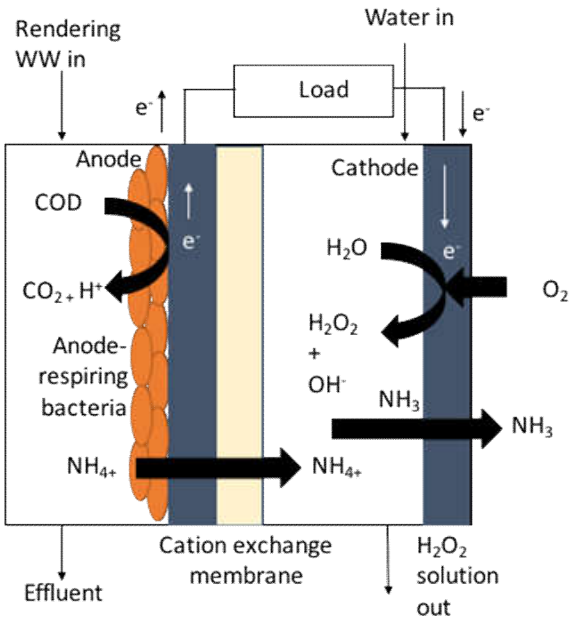

Figure 1. A schematic of the proposed concept for rendering wastewater (WW) treatment in MFCs with nitrogen recovery and peroxide production.

The body of work conducted on MFCs in the last decade suggests that rendering wastewater could potentially be an ideal anode feed for treatment. Also, MFCs will be scaled up as modular systems, and thus are suitable for deployment at smaller wastewater treatment plants, such as on site for rendering facilities. The main advantage that MFCs would bring to the rendering facilities would however be in terms of energy savings for wastewater treatment, recovery of N, and production of peroxide. The latter could especially be of importance in a rendering facility, as peroxide produced on site through wastewater treatment could be used for cleaning, or disinfection of the wastewater, or control of odors, which represents a major environmental issue for rendering facilities as well.

Materials and Methods

To demonstrate the applicability of MFCs for rendering wastewater treatment, we originally divided our proposed work into four specific objectives. Herein we describe the materials and methods used for each objective.

Objective 1:

Objective 1 ws divided into two parts. For the first part, we wanted to determine the anaerobic biodegradability of rendering wastewater. Such studies are typically done using what is referred to as biochemical methane potential (BMP) tests.

The BMP tests were carried out in 160 mL serum bottles. The number of bottles were selected based on the different ratios chosen on volume of rendering wastewater to an anaerobic digester sludge inoculum (in triplicates for each ratio chosen). Sample mixtures based on the composition were poured in beakers and stirred to ensure well-mixed samples. 100 mL volume of mixtures of rendering wastewater and inoculum were added to the serum bottle using a pipette. All the serum bottles were prepared after keeping the required number of septa and aluminum seal caps ready. The samples were purged in the serum bottles for 5 minutes to ensure anaerobic environment. After sparging, the septum was immediately placed on the serum bottle and followed by the aluminum cap which was sealed with the help of a crimper. Once all the bottles were sealed, they were placed in the temperature-controlled shaker at maintained at 37 deg C and 130 rpm. The volume measurement for the gas collected was done at the same time every day. To measure the volume, the bottles were placed in a chemical fume hood, and 20 mL or 50 mL frictionless syringes were used along with 23 G disposable needles to collect and measure gas volume. The amount of biogas collected was recorded in a spreadsheet and graphs of daily biogas generation (mL) vs. time (days), and cumulative biogas generation (mL) vs. time (days) were plotted.

The amount of methane generated was measured using a gas chromatography unit equipped with a thermal conductivity detector (Shimadzu GC 2014 with TCD) and a Restek ShinCarbon ST Micropacked Column (1.00mm x 1/16" x 2m). 0.25 mL of sample was taken from the headspace of the serum bottles using a Hamilton Gastight® GC Syringe. Ultra-high purity Argon was used as the carrier gas at 415 kPa pressure and 10 mL/min flowrate. The duration of run for each sample was set at 5 min, with the injector and the detector temperature set at 150

deg C whereas the column at 120 deg C. The area under methane peak in the chromatogram generated was used to calculate the percentage and concentration of methane generated. Graphs similar to daily and cumulative biogas generated were plotted for methane percentage.

An HPLC unit equipped with Aminex® HPX-87H Ion Exclusion Column (300 mm x 7.8 mm; 5 mM H2SO4, 0.6 mL/min) at 210 nm wavelength, was primarily used for analyzing the composition of volatile fatty acids that are formed during the operation of the lab-scale batch and semi-continuous digester. The duration of the run for each sample was set at 50 min and the temperature for the column was 30 deg C. 1 mL sample was drawn from the 20 mL collected using a 3 mL disposable luer lock syringe. The sample was filtered using a PVDF syringe filter with a pore size of 0.2 μm. 0.15 mL of the filtrate was taken in a 12x32 mm screw thread clear vial using a pipette and was diluted 10 times to have a total HPLC sample volume of 1.5 mL. An open-top polypropylene screw cap with 8mm Teflon septum was used to seal the vial. The samples were stored in the freezer until the analysis was carried out at the end of the week.

For the second part, we sought to determine if current production can occur from high acetate concentration wastewaters. For this, we set up four microbial electrolysis cells (MECs). Each MEC contained carbon rods as anode and cathodes and was made from a 300 mL glass bottle. No membrane was used to separate the anode and the cathode, and so all systems were single-chambered. The MECs were fed with synthetic wastewater containing 25 mM acetate and were inoculated with an anaerobic digester sludge. The MECs were run in batch mode, and current produced was monitored over time with a set anode potential of -0.1 V vs. SHE, a voltage shown in literature to result in current production from acetate.

Objective 2:

Objective 2 dealt with operating an MFC continuously to see what kind of nitrogen recovery rates we get through the transport of NH_4^+ through the cation exchange membrane to the cathode and subsequent volatilization to NH_3. Unfortunately, the rendering wastewater we received on three different occasions did not have high protein or NH_3 concentrations, and thus was not evaluated for nitrogen recovery. This should be a topic of further study in a future project.

Objective 3:

For Objective 3, we wanted to improve peroxide production in MFC cathodes. We chose to study cathode catalyst loading as an important parameter, on the basis of previous literature. MFC cathodes are made from a gas diffusion layer (GDL). The loading of the catalyst on the GDL would likely affect peroxide production. Although catalyst loading studies had been done in literature, we were not aware about any studies with GDLs. We used Vulcan carbon as the catalyst. We discuss below how we performed these studies.

Three different catalyst loadings for gas-diffusion cathodes were tested to determine cathodic Coulombic efficiencies. To prepare the cathodes, three square pieces of CeTech Carbon Cloth (Fuelcellstore.com) with dimensions of approximately 6 cm x 6 cm were cut out. CeTech Carbon Cloth is a woven carbon cloth with a microporous layer (MPL) that acts as a gas diffusion layer on one side, giving it a total thickness of 410 um. A square of approximately 5

cm x 5 cm in the center of each cloth was coated with the appropriate amount of Vulcan carbon catalyst ink, corresponding to Vulcan carbon catalyst loadings of 0.5, 1.5, and 3.33 mg/cm^2. The bare GDL was a 6 cm x 6 cm square of CeTech Carbon Cloth without any Vulcan carbon ink applied.

The carbon catalyst ink was prepared by adding 0.5 g of Vulcan XC 72R carbon powder to 1 mL of deionized (DI) water in a sterile 20 mL scintillation vial. Then, 5 mL of Nafion ® D-521 dispersion (5% w/w in water) was added to the bottle, and the mixture was placed in an ultrasonic bath for 30 minutes. The ink was then placed on a magnetic stirrer at a speed of 300 rpm for 24 hours. The final mass of the ink was 5.94 g.

The carbon catalyst ink was dispensed onto the carbon cloths by pipetting 0.15 mL, 0.45 mL, and 1.0 mL (corresponding to Vulcan carbon loadings of 0.5 mg/cm^2, 1.5 mg/cm^2, and 3.33 mg/cm^2, respectively) volumes of ink onto the carbon cloth, on the side opposite to the MPL, and spreading them with a paintbrush. The cathodes were allowed to dry overnight before testing began.

A cross-section of each cathode was imaged at the Clemson University Electron Microscopy Lab in Anderson, SC using a high resolution S4800 SEM. Samples of approximately 2 cm x 2 cm were first clamped between two glass microscope slides, with half of the sample in between the slides and half sticking out. The clamped samples were placed in liquid nitrogen for approximately 10 minutes. This helped freeze the fibers of the cloth in place so that they did not bend or shift when cut. Then, a razor blade was used to cut off a slice off the piece of the cloth that was sticking out of the glass slides, which was then discarded. The piece of the cloth that was sticking out of the glass slides was then mounted on a stub using double sided tape, with the edge that had been sliced with the razor oriented towards the electron beam. Each sample was imaged at 500X, 1000X, and 2000X.

A laboratory X-ray CT system, VECTor4CT (MILabs, The Netherlands) was used to obtain 3D images of all cathodes. Samples for imaging were prepared as 1 mm x 1 mm squares. Each sample was placed between foam in a horizontal tube that was inserted into the CT system. A source voltage of 55 kV and a current of 0.27 mA was used for each sample. A 3D reconstruction of each cathodes was performed using ImageJ, along with the Volume Viewer plugin. All images were first processed via automatically correcting threshold. To determine the porosity of each cathode, 10 cross-sectional images located 10 μm apart were taken from each 3D reconstruction in the X-Z direction. These images were further processed using MATLAB. The carbon within each image was set to appear as pink, and empty pore space was set to appear as black. MATLAB was used to count the number of pink and black pixels within each image, and the porosity of each cathode was calculated by finding the cross-sectional area of these pores and extrapolating them a distance of 10 μm. The porosity of each cathode was then calculated as the average of the 10 values.

Two identical electrochemical cells were constructed for this research. The electrochemical cells were made with three 10 cm x 10 cm plexiglass plates, as seen in Figure 2. Two of the plates had a 5 cm x 5 cm hole cut out in the center. The plate that did not have a hole in the center served as a backplate for the anode chamber. The plate with the hole that served as the anode

chamber was 1.3 cm thick, and the plate with the hole that served as the cathode chamber was 0.5 cm thick. Gaskets were used as necessary between the plates to prevent leaks. The anode and cathode chambers were separated by a CEM, which was chosen because it is less susceptible to peroxide degradation than anion exchange membranes (AEMs). The membrane used was a Chemours Nafion membrane (Fuelcellstore.com) and had a thickness of 127 um. The final volumes of the anode and cathode chambers were about 40 mL and 20 mL respectively, although these volumes varied slightly between experiments due to the flexibility of the membrane.

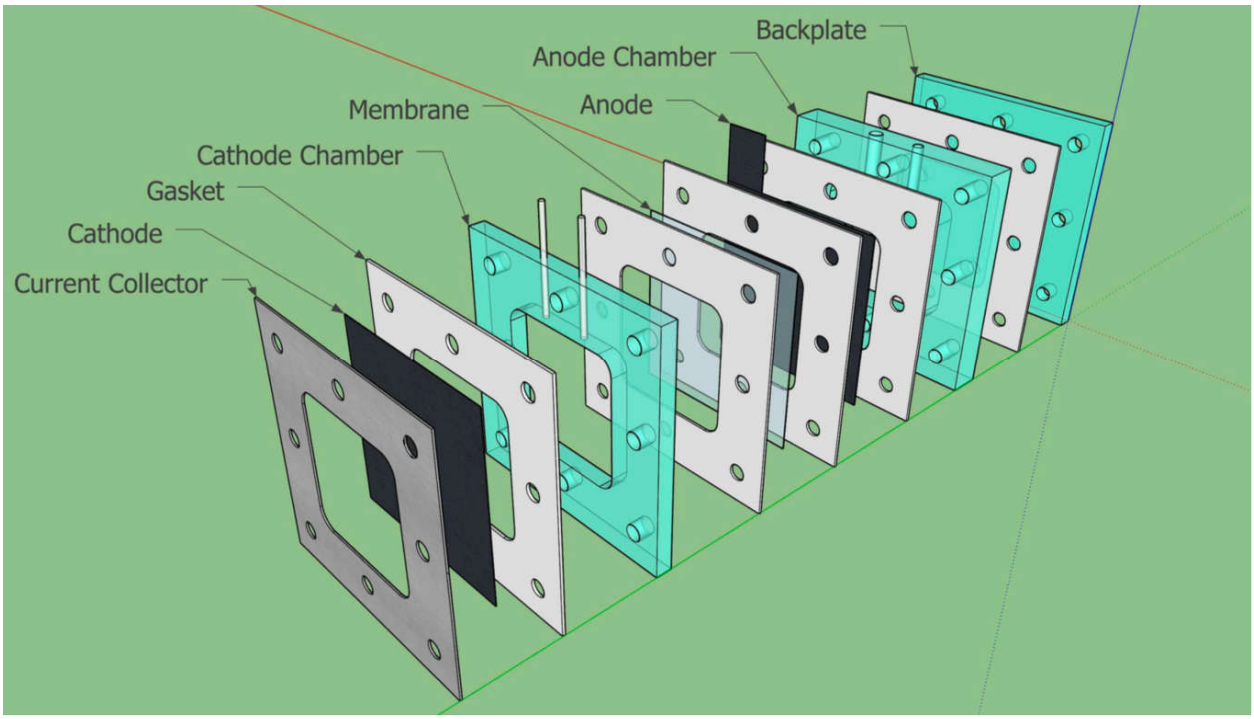

Figure 2. Expanded view of the electrochemical cells used for Objective 3.

Each chamber of the electrochemical cell had two holes in the top. The holes in the anode chamber allowed for the insertion of a reference electrode and for filling the chamber with a conductive salt solution. The holes in the cathode chamber were created to allow for a recirculation line to keep the cathode chamber well mixed for the duration of the H2O2 production experiments. The cathode chamber was contained by the membrane on one side and the cathode on the other side. The MPL of the cathode faced outside of the reactor to allow air to diffuse into the cathode chamber, and the Vulcan carbon catalyst-coated layer of the cathode faced into the cathode chamber. The anode was the same carbon cloth used for the cathode, but did not have an MPL. The layers of the electrochemical cell were held together with eight screws and wingnuts.

The reference electrode used was an RE-5B Ag/AgCl reference electrode with flexible connector from Bioanalytical Systems, Inc. It was inserted into a hole at the top of the anode chamber. For each experiment, the anode chamber was filled with a 100 mM phosphate buffer solution (PBS). The cathode chamber was filled with the same 100 mM PBS. The solution was pumped into the cathode using a Masterflex ® pump and the exact volume contained within the cathode chamber and the tubing was recorded at the start of each experiment. The cathode volumes ranged from

19-21 mL. Once the chamber was full, recirculation began by setting the pump speed corresponding to a recirculation rate of 25 mL/min. The recirculation line was intended to ensure the contents of the cathode chamber were well-mixed, and since the volume of the cathode chamber was about 20 mL, the slightly higher value of 25 mL was chosen to allow for recirculation of the entire chamber each minute. The electrochemical cell was connected to a BioLogic VMP3 Multi-Channel Potentiostat. EC-Lab software was used to set a constant current to the reactor. Each cathode was tested at currents of 2.5, 12.5, and 25 mA for 4 hours, corresponding to current densities of 0.1, 0.5, and 1 mA/cm^2 across a 25 cm^2 cathode. Each cathode/current combination was run in triplicate.

Linear sweep voltammograms (LSVs) were performed at the start of each experiment, before beginning peroxide production tests, to examine differences in polarization curves between the control and the cathodes with different catalyst loadings.

After the LSVs were performed, Chronopotentiometry (CP) method was used to apply constant current to the electrochemical cells. Once the current was set using EC-Lab software, liquid samples were taken from the cathode every 30 minutes. To take the samples, the pump was turned off and the tubing was disconnected from one of the ports in the cathode. A volume of approximately 0.25 mL was extracted at each time point, which allowed for two peroxide concentration measurements at each time point (a volume of 0.1 mL was required for each measurement, as described below in Section 3.1.8). The exact volume extracted from the cathode was recorded and accounted for in subsequent peroxide concentration calculations, because in a cathode of approximately 20 mL, removing even small volumes from the cathode produced significant differences in concentration calculations. The tubing was then reconnected to the cathode and the pump was turned back on until the next sampling point. At the end of each 4-hour experiment, the pH of the anode and cathode chambers were measured using a Thermo Scientific Orion STAR A211 pH meter. Additionally, the concentration of peroxide in the anode chamber was measured at the end of each experiment to ensure that the membrane was functioning properly and had not been damaged.

A standard curve of absorbance vs. peroxide concentration was produced by adding 0.1 mL volumes of known concentrations of peroxide to 1 mL of Titanium(IV) oxysulfate-sulfuric acid solution (27-31% H_2SO_4 basis) and 0.9 mL of DI water in a 2 mL plastic cuvette. The concentrations of peroxide used to create the standard curve were 0, 50, 100, 150, and 200 mg/L. The cuvettes were covered in parafilm and inverted twice, and then allowed to sit for 10 minutes while the reaction occurred. Then, their absorbance was measured in a VWR UV-1600PC spectrophotometer at 405 nm which had been blanked with a cuvette containing 2 mL of DI water. The average of the two absorbance measurements at a time point were used to calculate peroxide concentration at that time. Higher concentrations of peroxide resulted in darker yellow colors developing in the cuvettes.

Objective 4:

Work for Objective 4 was conducted as this project was ending and the next project on scale-up to L-scale MFC began, during Summer 2018. Since the design developed for Objective 4 is to be used as a benchmark for activities for the new project, the materials and methods for

Objective 4 are discussed in the progress report for the new project, rather than in this final report.

Results and Discussion

Anaerobic COD conversion from rendering wastewater

Over the duration of the project, we obtained rendering wastewater on three occasions and used it in BMP tests. During the three times we collected rendering wastewater, the total COD and LCFA concentrations varied, but the composition did not. We have detected predominantly C16 and C18 saturated and unsaturated LCFAs. We have also detected a significant concentration of acetate in rendering wastewater (results not shown).

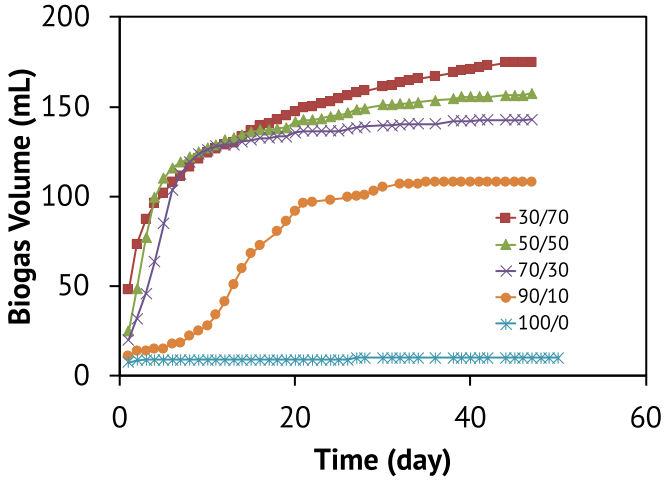

Figure 3. Cumulative biogas production in BMP tests with rendering wastewater. Ratios shown are rendering wastewater/inoculum ratios by volume.

Our first goal was to demonstrate the anaerobic biodegradability of rendering wastewater COD. We did this through BMP tests, where we track how much methane is produced from rendering wastewater. In an MFC, the methanogenic step is replaced by current production, but measuring methane allows to determine the maximum possible COD conversion we could potentially achieve in MFCs. In Figure 4, we show total biogas production from rendering wastewater, when incubating different ratios of wastewater to inoculum. Incubation of rendering wastewater by itself did not result in any biogas production, suggesting an absence of methanogens in rendering wastewater. For rendering wastewater/inoculum ratios of 30/70, 50/50 and 70/30, we see no distinct differences in biogas production, whereas for 90/10, we see a lag in biogas production, likely from inhibition of methanogens from presence of LCFAs. We anticipate that such inhibitions would not be as pronounced in MFCs, as ARB grow as a biofilm where they may be protected from

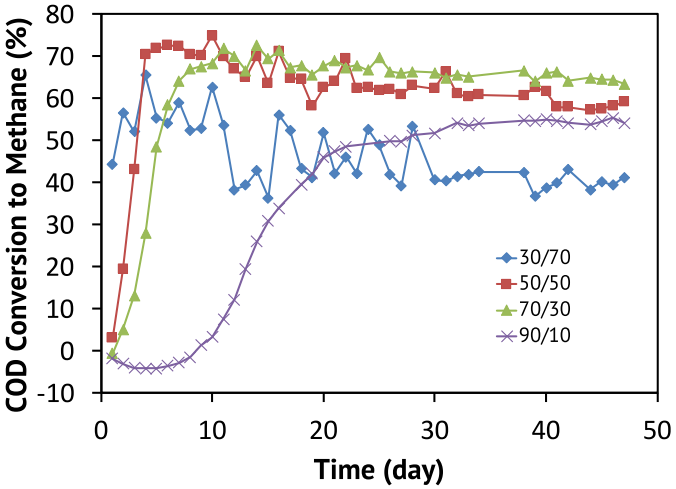

Figure 4. COD conversion to methane during BMP tests. Ratios shown are rendering wastewater/inoculum ratios by volume.

high concentrations of LCFAs.

In Figure 4, the BMP data is converted to COD conversion values. Within 5 days of incubation, COD conversions for all rendering wastewater/inoculum ratios except 90/10 increased to 70%. This is promising and suggests that high conversion of waste LCFAs to the final end-product is possible under anaerobic conditions. In Figure 5, acetate concentrations, as produced from beta-oxidation of LCFAs are shown. No other VFAs were detected; although when fats are co-digested with sludge at municipal treatment plants, other VFAs are often detected. This is likely due to a simpler composition of rendering wastewater.

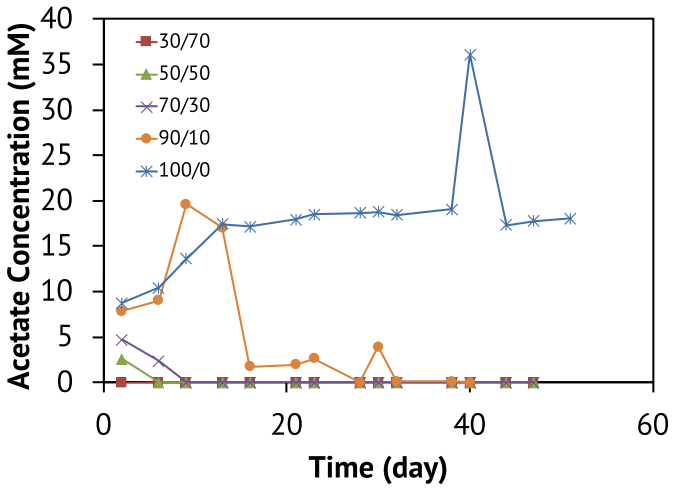

Figure 5. Profile of acetate concentration during BMP tests.

For the 90/10 ratio, acetate accumulated for a few days prior to its decrease, confirming an inhibition on methanogens, whereas in the rendering wastewater only incubation (100/0), acetate was produced but never consumed. The fact that acetate production was observed in this incubation suggests that the rendering wastewater likely contains beta-oxidizing microorganisms.

Electrical current production from high acetate concentrations

Shown in Figure 6 is the profile for electrical current production in one of the MECs fed with 25 mM acetate. A total of four MECs were operated and all followed the same trend. The electrical current production trend is similar to that observed for MECs in general that are inoculated with anaerobic digester sludge. Over the first three days there is not current production; this is due to cells predominantly just attaching to the electrode rather than actively growing. Between days 3 and 5, there is an exponential increase in current resulting from growth of anode-respiring bacteria on the electrode. During this phase, the doubling time was noted as 8 hours. Once 1 A/m^2 is reached the biofilm does not grown exponentially any more, likely because of some inhibition, but still overall high of 4 A/m^2 is reached after about 20 days, and then maintained thenceforth. In a continuously-fed MEC, we would thus expect to see these levels of current production from the concentrations of acetate found in rendering wastewater.

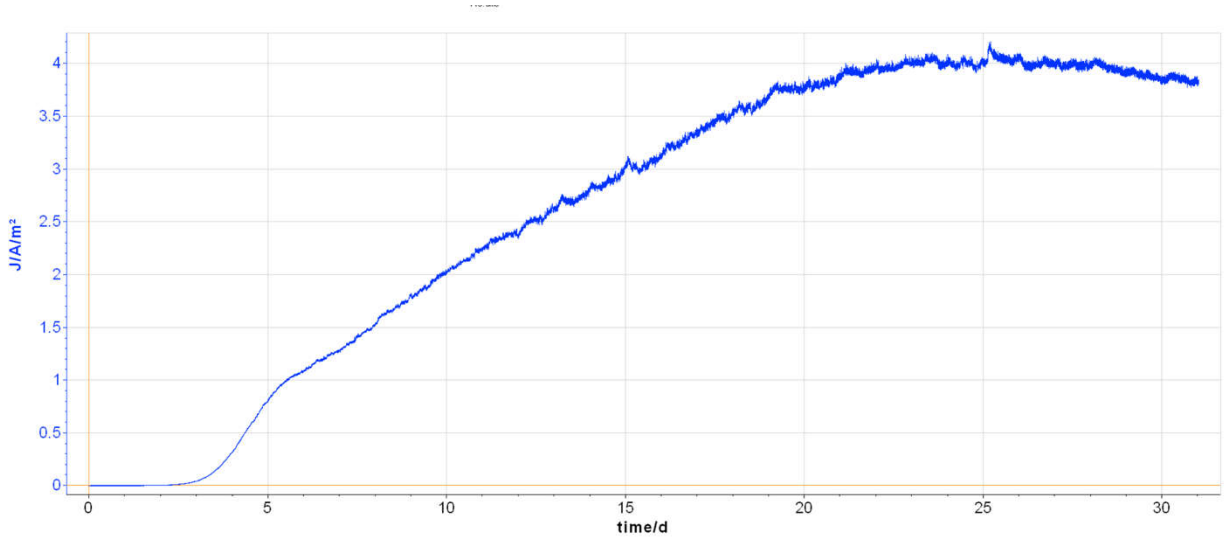

Figure 6. Electrical current production from 25 mM acetate solution in an MEC. The poised anode potential was -0.1 V vs. SHE.

Effect of cathode catalyst loading on peroxide production

Previous studies on fuel cells electrodes have shown that peroxide production on cathodes is dependent on the catalyst loading. In chemical fuel cells, peroxide is an unwanted product, but in this case, we want to produce peroxide.

Consistent with previous studies, lower catalyst loadings on gas-diffusion cathodes also generally produced higher peroxide concentrations, resulting in higher cathodic Coulombic efficiencies, as seen in Figures 5a and b. The 0.5 and 1.5 mg/cm^2 cathodes performed very similarly to each other at all current densities tested, while the 3.33 mg/cm^2 cathode consistently produced lower concentrations and thus lower efficiencies than the other cathodes.

Figures 7a and b show the average peroxide concentrations and cathodic Coulombic efficiencies at a current density of 1.0 mA/cm^2. The 0.5 and 1.5 mg/cm^2 cathodes had similar production efficiencies of 26-65% and 27-60%, respectively, whereas the 3.33 mg/cm^2 cathode had much lower production efficiencies ranging from 21 to 33%. The trends are similar, with the 0.5 and 1.5 mg/cm^2 catalyst loadings producing similar peroxide concentrations to each other, while the 3.33 mg/cm^2 catalyst loading produced much lower concentrations than all other cathodes. A control experiment was also performed at the highest current density a bare GDL, which surprisingly produced the highest peroxide concentrations and the highest Coulombic efficiencies.

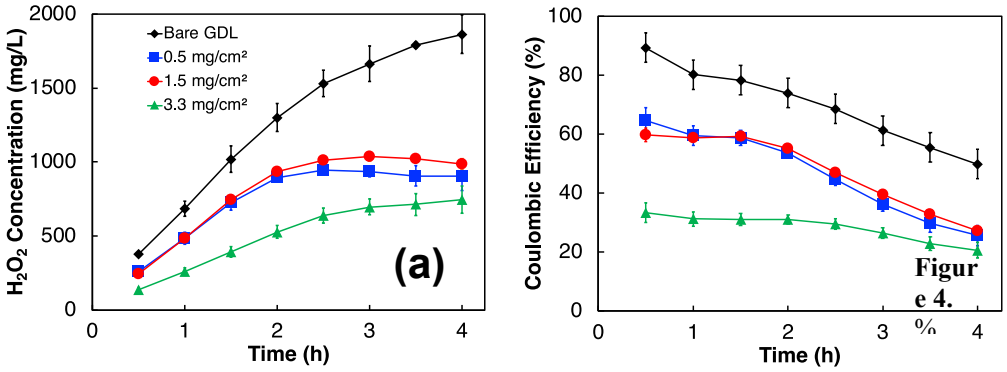

Figure 7. (a) Peroxide concentrations and (b) cathodic Coulombic efficiencies for three different catalyst loadings at a current density of 1 mA/cm2. All concentrations and efficiencies are the averages of three replicates. Error bars represent one standard deviation. The control was a piece of carbon cloth with no Vulcan carbon catalyst ink.

The results shown above raise an important question. If the bare GDL produced the highest cathodic Coulombic efficiencies and peroxide concentrations, then there may not be a need for the application of a Vulcan carbon catalyst at all. However, there is another benefit to adding the carbon catalyst ink. Figure 8 shows the voltammograms for each of the cathodes, along with that of the bare GDL. Clearly, the bare GDL shows a much higher overpotential than any of the cathodes coated with the catalyst. Therefore, the control cathode would not allow for the production of peroxide at reasonable current densities, while achieving energy neutral or positive treatment in the overall MFC system.

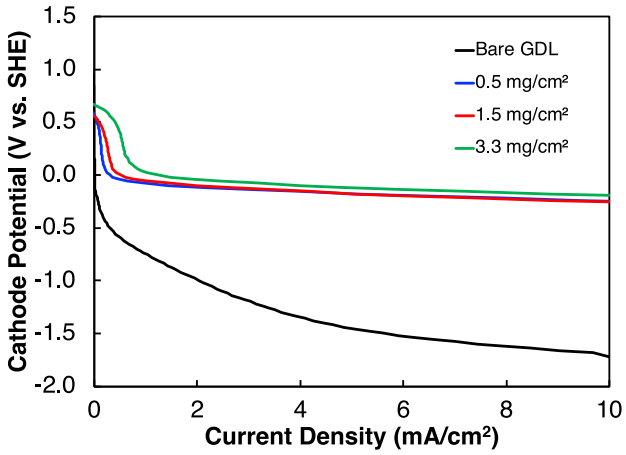

Figure 8. Voltammograms of the 0.5, 1.5, and 3.33 mg/cm^2 cathodes and the control at room temperature and an ionic strength of 100 mM.

It appears that the addition of any amount of Vulcan carbon catalyst causes a dramatic decrease in the overpotential of the cathode. The lowest overpotential was produced by the highest catalyst loading. Again, as in the peroxide production results, the 0.5 and 1.5 mg/cm2 cathodes show very similar results. Therefore, although the bare GDL had the highest cathodic Coulombic efficiencies, the application of the carbon catalyst is still justified for the dramatic decrease in overpotential.

The SEM images showed that different loadings of carbon catalyst ink did not lead to different layer thicknesses on the cathodes as initially expected. Figure 9a shows a cross section of the highest catalyst loading at a magnification of 100X. If the carbon catalyst ink formed a distinct layer on top of the carbon cloth, then intuitively, the highest loading would have the most noticeable layer of any of the cathodes. However, although the individual fibers of the carbon cloth can be identified from this image, there is not a distinct layer of ink sitting on top of the

cloth. Images at a higher magnification reveal more about the differences in distribution of the carbon ink on the different cathodes. Figure 9b show cross-sectional images of the bare GDL, 0.5, 1.5, and 3.33 mg/cm^2 cathodes, respectively, at a magnification of 1000X. Although the cloth fibers and the ink are both made of carbon, there are clear differences in morphology that allow us to determine the location of the Vulcan carbon catalyst. The fibers of the control, which had no Vulcan carbon catalyst applied, appear very smooth. Each of the prepared cathodes show small pieces of Vulcan carbon that appear to coat the individual fibers of the cloth in a relatively uniform way. This image shows that in contrast to what was expected, the ink seeps through the entire thickness of the cloth instead of sitting on the top layer of the woven fibers. The 0.5 and 1.5 mg/cm^2 cathodes appear much more similar in terms of how much ink coats the fibers, as opposed to the 3.33 mg/cm^2 cathode, which shows much larger clumps of ink and much less empty pore space. This is not surprising, as the 0.5 and 1.5 mg/cm^2 cathodes performed similarly in the peroxide production experiments and the voltammograms as well.

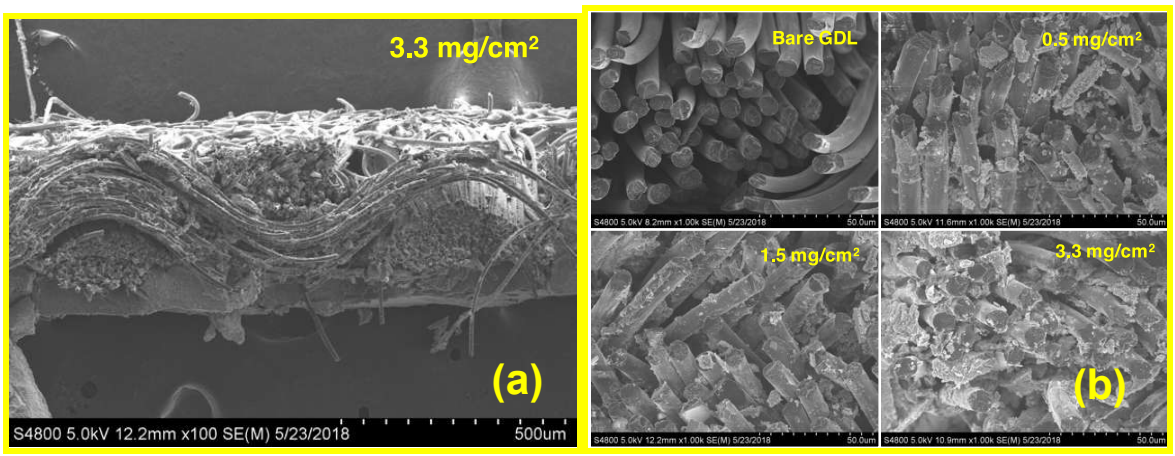

Figure 9. a) Cross section of the 3.33 mg/cm^2 catalyst loading at a magnification of 100X. The side that was coated with carbon ink is at the top of the photo. The image was created using the S4800 SEM at the Clemson University Electron Microscopy Lab. b) Cross-sectional SEM images of different catalyst loadings at 1000X magnification.

Examination of these images led to a hypothesis. For the peroxide concentrations to be measured in the cathode chamber, the peroxide must diffuse through the cathode and enter the electrolyte. The lack of empty pore space in the 3.33 mg/cm^2 cathode may cause a more tortuous path for the peroxide in this cathode. This may lead to the formation of local pore spaces accumulating higher concentrations of peroxide than the bulk electrode, where under alkaline conditions, the accumulation of HO_2^- ions leads to new decomposition pathways, degrading H_2O_2 to water, oxygen, and OH^- ions. Thus, the lack of pore space with higher catalyst loadings may result in higher consumption of the H_2O_2 within the fibers themselves, which ultimately leads to lower concentrations of H_2O_2 measured in solution.

To confirm this, porosity measurements were made using X-ray CT scans. Figure 10 shows the 3D reconstructions of each cathode. When applied for fuel cell GDLs and membrane electrode assemblies, X-ray CT scans allowed for reconstruction of each separate layer in the system, primarily because the material used for each layer was different. In this research, a GDL consisting of carbon cloth with a carbon MPL on one side and a Vulcan carbon catalyst ink coating on the other side was used. Due to the moderate resolution of the CT system used, 10

μm, and the fact that all layers were composed of carbon with a fairly similar density, it was not possible to segment each layer separately.

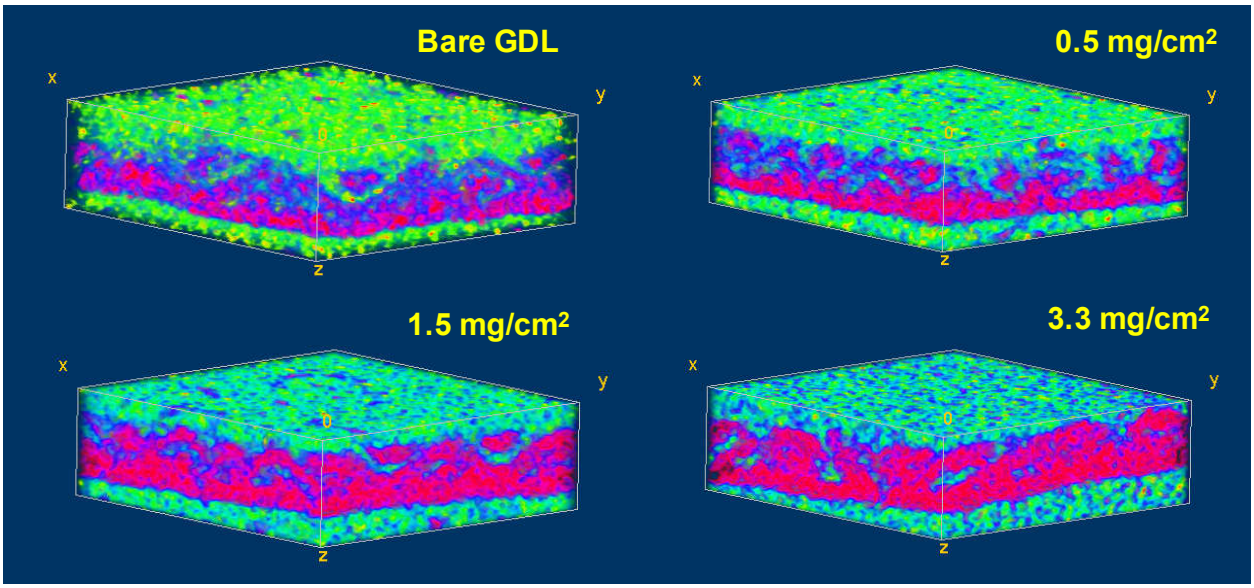

Figure 10. 3D reconstructions of each cathode (a) bare GDL, (b) 0.5 mg/cm^2, (c) 1.5 mg/cm^2, and (d) 3.33 mg/cm^2, obtained via the Volume Viewer plugin on ImageJ. The green color shows the foam support, whereas the pink color shows the location of carbon.

However, Figure 10 does show that increasing the carbon loading on the electrodes leads to more carbon seeping through the cloth, which is also shown by the SEM images discussed above. On all images, there is a layer of dense carbon at the bottom of each cathode, representing the MPL. Throughout the rest of the cathode, increasing carbon loading leads to increased carbon detected through X-ray CT, instead of just on the top, as one would expect for a typical GDL. The GDL used in this research, CeTech carbon cloth, has a 30% PTFE treated cloth, and these images imply that this treatment is low enough to allow the ink to seep through during the coating process.

The 3D reconstructions were processed further using MATLAB to determine the porosity of each cathode. A total of 10 segments per cathode were used for porosity determinations, which are shown in Table 1. The porosity determinations show that the bare GDL has a porosity of 66.9%, while each of the other cathodes have a decreasing porosity with increasing Vulcan carbon loading. Thus, instead of increased catalyst thickness, the higher catalyst loadings in these experiments manifest as decreased porosity of the cloth layer. As discussed above, lower porosity leads to higher diffusional resistance for peroxide out of the catalyst layer, likely affecting the overall cathodic Coulombic efficiency.

Table 1. Porosity (%) of each cathode sample obtained via image processing on 3D reconstructions. Ten segments were used for each porosity determination, and the average values are reported along with standard errors.

	Porosity (%)
Bare GDL	66.9 ± 0.7
0.5 mg/cm²	40.7 ± 1.3
1.5 mg/cm²	38.0 ± 1.3
3.3 mg/cm²	31.8 ± 0.4

Impacts and Significance:

The impacts, both in terms of environmental sustainability and economic benefits for rendering facilities, of a successful development of MFCs for rendering wastewater treatment, are immense. MFCs will help achieve many of the research outcomes highlighted in the survey conducted by Fats and Proteins Research Foundation on research needs for rendering facilities.

First, MFCs represent an energy efficient method for treating rendering wastewater. Compared to aerobic biological treatment that requires significant energy input for aeration, MFCs rely on anaerobic processes to convert COD to electrical current. Thus, there is a significant reduction in the energy required and cost of secondary treatment. This outcome will directly meet the research goal 1.47 (increasing efficiency and effectiveness of wastewater treatment). Second, although the MFCs studied here did not specifically evaluate nitrogen recovery, they could help recover nitrogen from rendering wastewater. N is recovered in the form of NH_3 gas, which can be re-dissolved in acid to produce NH_4Cl. The conditions at the cathode could also be tailored to result in phosphorus (P) recovery as struvite as well. Both N and P recovered in useful forms represent additional co-products for the rendering industry. Thus, MFCs could help achieve the goal 1.79 (extracting phosphorus and other usable products from wastewater or sludge).

Finally, and perhaps most novel, is the production of peroxide at the cathode of MFCs. peroxide is an important chemical in the context of water/wastewater treatment. For example, peroxide can be used for disinfection or advanced oxidation. peroxide can be used for cleaning and sanitizing equipment. peroxide can also be used for removing odors via chemical scrubbing. Thus, peroxide production on site could be very useful. Overall, MFCs provide a unique opportunity in turning rendering wastewater into a source of additional co-products as well as producing important chemicals for using on site, helping achieve the goal 2.29 (environmental sustainability of rendering).

Publications:

Currently two journal submissions are in preparation, and expected to be submitted in the 2018-19 academic year:

1. Murawski E., Kananizadeh N., Popat S.C. Effect of cathode catalyst loading and current density on peroxide production in microbial fuel cells. To be submitted to *Environmental Science & Technology Letters*.
2. Xie A., Popat S.C. Comparison of rendering wastewater treatment in microbial fuel cells and methanogenic reactors. To be submitted to *Water Research*.

Outside funding:

The following proposal submissions on the topic of MFCs with peroxide production were made. Whether a grant was awarded or not is noted as well.

1. Microbial fuel cells with peroxide production for space life support systems applications. South Carolina Space Grant Consortium. Awarded $10,000.
2. Palmetto Academy: Microbial peroxide-producing cells for blackwater treatment during space missions. South Carolina Space Grant Consortium. Awarded $18,000.
3. Microbial peroxide-producing cells for blackwater and greywater treatment during space missions. South Carolina Space Grant Consortium. Awarded $20,000.
4. Combined advanced oxidation and biological treatment of landfill leachate in microbial peroxide-producing cells. Environmental Research and Education Foundation. Requested $151,973. Not awarded.
5. Electrochemical disinfection of drinking water enhanced through cathode-produced hydrogen peroxide. Environmental Protection Agency. Requested $50,000. Not awarded.
6. Microbial peroxide-producing cells as a landfill leachate pre-treatment technology to recovery ammonia and address emerging leachate organic matter issues. National Science Foundation. Requested $195,750. Not funded.

Future Work:

Future work on MFCs for rendering wastewater should include testing of modular L-scale reactors. This work was proposed as part of the ACREC program for the fiscal year 2018-19 and was awarded. Results from this scale-up effort will be presented in the next research review meeting in Spring 2019. If successful at the L-scale, the MFCs should be scaled to a larger size for an onsite evaluation at a rendering plant.

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