## Specific Aim 1: Test the effect of temperature and acetate addition on MFC power density and biofilm properties

### Rationale

Environmental microbial communities can have a diverse mixture of species filling different ecological niches. By enriching these diverse communities under operating conditions similar to their intended purpose, selective pressures shape the microbial community to become more efficient at deconstructing a given substrate. Determining the effect of MFC process variables on MFC performance is the first step in identifying the mechanism behind microbial community biofilm formation in MFCs and elucidating the optimized parameters for higher power density. Specifically, temperature and acetate addition during startup will be tested because of their ease of manipulation in a laboratory setting, their adaptability to an industrial process, and previous literature showing that 20 mM acetate-initiated MFCs display higher power densities once switched to their target substrate (Sun et al., 2015).

Fluorescence microscopy offers the opportunity to study specific spatial properties of the biofilms in microbial fuel cells and their differences across treatments, specifically thickness and coverage. Additionally, Illumina sequencing provides the ability to determine microbial community relative abundance of species and diversity. Performing both fluorescence microscopy and Illumina sequencing on the biofilms in MFCs will provide information on the mechanism of biofilm growth and phylogenetic diversity as a function of temperature and acetate addition.

### Experimental Approach

Bacteria will be extracted from the food waste anaerobic digester (AD) here at UC Davis, which my initial experiments have shown contain a microbial community capable of electrogenesis. This bacterial community will then be inoculated into MFCs according to a response surface design (central composite) with 3 operating temperatures x 3 different acetate concentrations added during startup. The three operating temperatures are chosen to be 30°C, 42.5°C and 55°C due to 30°C and 55°C being common temperatures for anaerobic digesters with one temperature in between to determine second order effects. Acetate concentration of 20 mM is chosen through existing literature as an optimal quantity (Sun et al., 2015) and 40 mM is chosen due to lack of knowledge in the literature on this factor level. 5 replicates will be added at the center point for an independent measure of population variance. This experimental design includes a total of 8+5=13 MFCs for treatment, plus one for control, plus one for validation. This is a total of 15 MFCs (see table below).

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  |  | MFC Power Metrics | | | Biofilm | | | | Microbial Ecology | | |
| run | type | temp | acetate | power\_density | COD\_reduction | coulombic\_efficiency | biofilm\_thickness | live\_dead\_ratio | live\_cells | dead\_cells | richness | evenness | diversity |
| 1 | corner | -1 | -1 |  |  |  |  |  |  |  |  |  |  |
| 2 | corner | -1 | 1 |  |  |  |  |  |  |  |  |  |  |
| 3 | corner | 1 | -1 |  |  |  |  |  |  |  |  |  |  |
| 4 | corner | 1 | 1 |  |  |  |  |  |  |  |  |  |  |
| 5 | edge | 0 | -1 |  |  |  |  |  |  |  |  |  |  |
| 6 | edge | -1 | 0 |  |  |  |  |  |  |  |  |  |  |
| 7 | edge | 0 | 1 |  |  |  |  |  |  |  |  |  |  |
| 8 | edge | 1 | 0 |  |  |  |  |  |  |  |  |  |  |
| 9 | center | 0 | 0 |  |  |  |  |  |  |  |  |  |  |
| 10 | center | 0 | 0 |  |  |  |  |  |  |  |  |  |  |
| 11 | center | 0 | 0 |  |  |  |  |  |  |  |  |  |  |
| 12 | center | 0 | 0 |  |  |  |  |  |  |  |  |  |  |
| 13 | center | 0 | 0 |  |  |  |  |  |  |  |  |  |  |
| 14 | validation1 | 0 | 0.5 |  |  |  |  |  |  |  |  |  |  |
| 15 | validation2 | 0 | 0.5 |  |  |  |  |  |  |  |  |  |  |

The control for this experiment will be an MFC with autoclaved substrates and inocula to ensure that COD reduction and power production are purely through biological activity. Positive control is not relevant at this point except for troubleshooting.

MFCs will be operated in batch mode. Acetate will be added to digestate at molar concentrations previously specified during feedstock addition. Power density responses at peak voltage after each feeding cycle will be measured with the Hach Colorimetry COD (Chemical Oxygen Demand) Assay and PicoLogger hardware to determine total energy output, chemical oxygen demand (COD) reduction, and maximum power density (W/m^2).

MFCs will be sacrificed after stable power generation for 3 cycles is reached on all units. At that time all units will be sacrificed. Powersoil DNA Extraction is a kit that extracts the DNA from the environmental sample. Additionally, I will take fluorescence microscopy micrographs of carbon cloth anode samples for the MFCs to measure biofilm thickness. Dyes used are propidium iodide (red) for staining ruptured membranes and SYBR Green I (green) for intact membranes. Calcofluor will be used to stain the biofilm exopolysaccharide layer.

### MFC Power Metric Measurement Power metrics including power density, COD reduction, and coulombic efficiency will be measured for each cycle. Power density will be measured at maximum voltage after refeeding after units have displayed consistent voltage measurements for three cycles. COD reduction will be measured by Hach Colorimetry both at the beginning of the cycle and at the end of the cycle. COD at the beginning of the cycle will be measured with stock solution COD and the ending COD of the last cycle in the MFC. COD will be calculated at the end of the cycle with Hach Colorimetry. Coulombic efficiency will be calculated using standard equations and COD reduction measurements.

### DNA Extraction and Microbial Ecology Measurement

DNA extraction will be performed after power density measurements have been performed for all units. At that time all units will be sacrificed. Three different materials will be sampled in each MFC – the carbon anode, the bulk liquid, and the air cathode. This will give sufficient DNA to determine community differences across all treatments. Bulk liquid will be sampled using the standard method of using 250 ul of liquid for DNA extraction. The carbon anode will be cut in half vertically, cutting from top to bottom, as will the air cathode. Half of each of these materials will be used for DNA sampling and half will be used for fluorescence microscopy. Both the carbon anode and air cathode will be cut and inserted into powerbead tubes extract biofilm, then this biofilm will be added to the Powersoil DNA extraction kit for extraction.

All samples will undergo quality control with Qubit, Biospec, PCR, and E-gel measurements recorded.

Samples (14x3=42) will be sent to RTL Genomics for characterization.

### 1.5 Biofilm Measurement

The other half of the carbon anode that was cut in half will be stained with calcofluor, propidium iodide, and SYBR Green I immediately after sacrifice. They will then be imaged with a fluorescent microscope. 14 carbon anodes will thus be imaged. For each carbon anode, they will undergo random imaging at 10 points for z stacks, resulting in 10 z stacks x 14 anodes = 140 images for analysis. Air cathodes will be stained, saved, and frozen for future potential analysis.   
  
TIMELINE

**Set up**

15 MFCs will be constructed

14 will be filled with centrifuged sludge (14,100 rpm for 1 minute)

1 will be filled with similarly centrifuged sludge then autoclaved

Acetate addition

Three units will have no acetate addition (coded -1). Units coded 0 will have 20 mM acetate addition, and units coded +1 will have 40 mM acetate addition. Quantity of acetate added will be calculated as (20 mmol/Liter) x (volume of each reactor in liters). Quantity for 40 mM will be similarly calculated.

Keep COD same between all treatments?

Three different incubators will be used to maintain the MFCs at 30C, 42.5C, and 55C. Units coded at -1 will be kept at 30C, units coded at 42.5C will be coded at 0, and units kept at 55C are coded at +1.

All units will be connected to a 1 kOhm external resistor? and connected to the PicoLogger for voltage measurement.

**Maintenance**

Each day units will be checked for liquid levels and topped off with DI water if there is significant liquid loss.

Each day Picologger will be checked for bugging out

When units drop below 10 mV, units will be refed with centrifuged sludge and the corresponding acetate concentration. COD measurement will be performed for the beginning and end of each cycle as previously stated.

Power density measurements will be performed for each unit after three successive cycles of consistent voltage production.

**Takedown**

After power density measurements have been performed for all units after their three successive cycles of consistent voltage production, all units will be sacrificed at once to control for time of biofilm growth. At this time each unit will be sampled for DNA and undergo fluorescence microscopy as previously specified in section 1.4 and 1.5.

Data will be analyzed according to the Qualifying Exam document.