Cantaurus, Vol. 27, 8-11, May 2019 © McPherson College Department of Natural Science

Relationship between bacterial community structure and the performance of microbial fuel cells in Kansas soil samples

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ABSTRACT

Microbial fuel cells (MFC) harvest energy from renewable resources which allows for obtaining clean and renewable energy. In this research soil samples from a freshwater and a salt marsh source were used as the source of energy for the microbial fuel cells. The bacterial community was analyzed using shotgun sequencing to find differences among the soil samples. The overall energy output from the MFCs were analyzed for thirty days to find which sample produced more energy. It was found that the samples did have two significantly different bacterial communities. The salt marsh samples produced more power over the trial period but was not significantly more than the freshwater samples. This suggest that over a longer period of time salt marsh samples may produce more power.

Keywords: *Microbial fuel cell, salt marsh, freshwater*

INTRODUCTION

Current clean energy options include wind, solar, fuel cells, and more. It has been shown that renewable sources of energy can reduce overall energy demand, system cost, and emissions (Dincer 2015). Fuel cells use chemical energy of fuels, like hydrogen, to produce electricity. One type of fuel cell is a microbial fuel cell (MFC). This type of fuel cell uses microorganisms that oxidize organic substrates and produce electricity in doing so. This type of fuel cell is relatively easy to produce and use, making it a viable source of clean energy.

Potter (1911) discovered that bacteria produce electricity, but the recent interest in new energy sources started the current research on how to maximize the energy harvested from these microbes. Specifically, factors that affect performance like inoculum, temperature, and substrates (Heidrich, et al 2018). These special bacteria produce electricity because of their ability to donate the final electron on the outside of the cell. This is done because of the anaerobic environment the cells are in. Not having oxygen as an electron acceptor allows the electron at the end of the transport chain to go to an alternative acceptor like directly to an anode. Electricity is being produced by the electron released from the organic substrate the bacteria is metabolizing. The energy being created is harvested by an MFC that includes an anode and cathode to create an electrical circuit. (Logan, et al 2006).

Research has found that species in the genera *Shewanella* and *Geobacter* are efficient in producing power within the MFCs (Kiely, et al 2011). MFCs with one isolated bacterial species can produce more power, but they can be expensive and difficult to maintain (Kiely et al 2011). It is much easier to gather a sample of soil that has a community of these species, along with others, and use the soil in the MFC. Understanding all the relationships between these communities and their substrates can be complex and few papers have studied the bulk parameters of the bacterial community (Dunaj, et al 2012).

The bacteria that produce electricity can be found usually in places that are anaerobic. This can include extreme environments, but also environments like water-saturated soils. The studies that have looked at the overall community within the soil samples have mostly looked at freshwater samples (Dunaj et al 2012). Salt marsh soil samples can also provide an apt bacterial community for MFC performance due to the hypoxic conditions created by decomposing plant material (NOAA). It has been shown that methanogenesis and sulfate reduction can happen simultaneously within estuarine soil, which can be comparable to salt marsh soils. (Oremland et al 1982). The anoxic conditions in salt marsh soil and the ability to perform methanogenesis and sulfate reduction selects for bacteria that can produce electricity and can metabolize a variety of substrates compared to fresh water.

In this research, the bacterial community structure, MFC performance, and soil characteristics in MFCs constructed from freshwater and salt marsh soils found in various areas within Kansas are studied in order to understand how soil type and bacterial community influence MFCs.

MATERIALS AND METHODS

Soil Sample Collection

Soil samples were collected from two sites. The salt marsh sample was collected from the Quivira National Wildlife Refuge, located in south central Kansas near the town of Stafford. The fresh water sample was collected from the Little Turkey Creek in McPherson, KS. The sampling location at each site was selected

based on ease of access.

Microbial Fuel Cell Construction

The MFC's were developed by a company named Magical Microbes. This company produces MFC kits called a Mudwatt. This kit comes with a round cathode and anode carbon felt and respective wires, blink board, capacitor (10μF), LED, a vessel and a circuit board where the wires of the anode and cathode connect and where the capacitor and LED light connect. This goes on top of the vessel lid and turns the low voltage and low current into short bursts of high voltage and current causing the LED light to blink.

Both the salt marsh and freshwater soil samples were homogenized respectively. A sample of the soil was then put into its respective vessel up to 1 cm mark. The anode was then placed on top and pressed into the soil, ensuring there were no air bubbles and completely saturated with water. Deionized water was added if needed. Then more soil was added on top of the anode to the 5 cm mark. Again, it was packed to ensure all air bubbles were out. The cathode was gently placed on top and exposed to air.

The circuit board is attached to its designated spot on the lid of the vessel. The anode and cathode wires were threaded through the holes in the lid of the vessel and the cathode was plugged into the "+" port on the circuit and the anode into "-" port on the circuit board. The long end of the capacitor was connected to Pin 1 and the short end to Pin 2. The LED's long end was connected to Pin 5 and the short end to Pin 6. The lid was then sealed onto the vessel with tape around the edges to try to prevent water loss. This was done for 20 samples of the salt marsh soil and 20 samples of the freshwater soil, creating 40 MFCs total.

Power Output Analysis

The electrical current was analyzed using iWorx program. It was attached to the hacker board using alligator clips to measure the timing of the voltage spikes. Statistical analysis was done with the program SigmaPlot.

Bacterial Community

The samples of the soil were sent off to CD Genomics where shotgun metagenomic sequencing was performed. The samples were then processed and analyzed producing a large amount of information including gene prediction, taxonomy annotation, function annotation, antibiotic resistant genes analysis, and more.

The metagenomic sequencing method began with sample testing. Three methods of quality control for the DNA samples were used: DNA degradation degree and potential contamination was monitored on 1% agrose gels, DNA purity was checked using the NanoPhotometer spectrophotometer, and DNA concentration was measured using Qubit dsDNA Assay Kit in Qubit 2.0 Flurometer. DNA contents above 1 ug were used to construct library.

For library construction a total amount of 1μg DNA per sample was used as input material for the DNA sample preparations. Sequencing libraries were generated using NEBNext® UltraTM DNA Library Prep Kit for Illumina (NEB, USA) following manufacturer's recommendations; and index codes were added to attribute sequences to each sample. Briefly, the DNA sample was fragmented by sonication to a size of 350bp,then DNA fragments were end-polished, A-tailed, and ligated with the full-length adaptor for Illumina sequencing with further PCR amplification. At last, PCR products were purified (AMPure XP system) and libraries were analyzed for size distribution by Agilent2100 Bioanalyzer and quantified using real-time PCR.

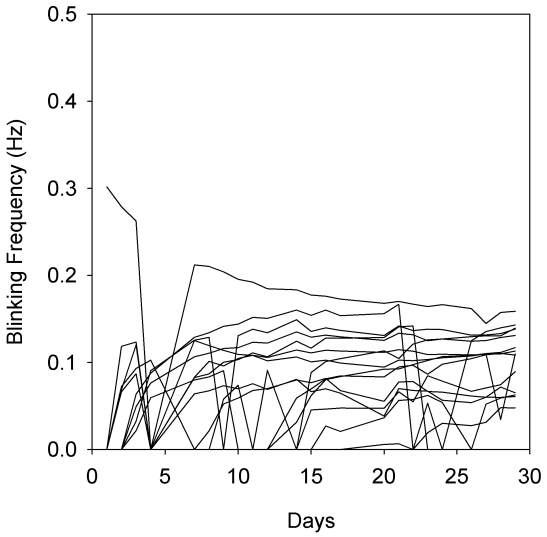
For sequencing the clustering of the index-coded samples was performed on a cBot Cluster
Generation System according to the manufacturer's instructions. After cluster generation, the library preparations were sequenced on an Illumina HiSeq platform and paired-end reads were generated.

RESULTS

Power Output

The total power output of the freshwater samples was 6.22×10^{-4} watts and the salt marsh total output was 9.34×10^{-4} watts. A two-sample t-Test assuming equal variances produced a p-value of 0.06452469, which is above the threshold of 0.05 for significance.

a.



b.

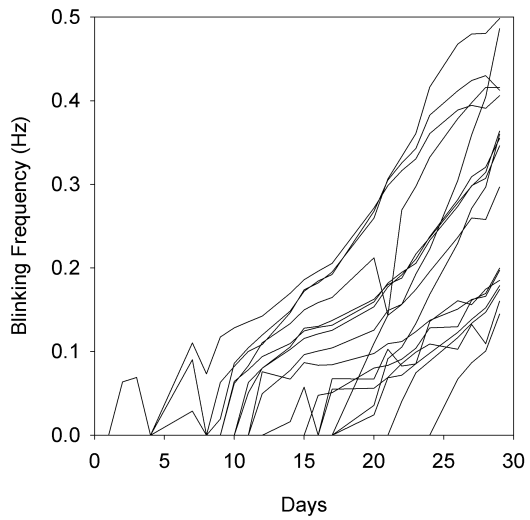
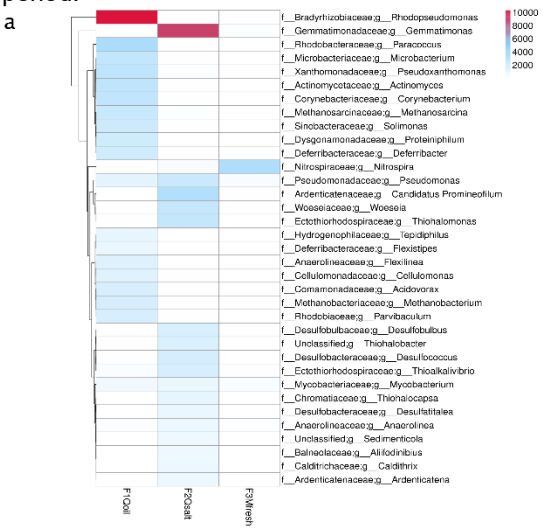


Figure 1. a. Freshwater frequencies over the 30-day period. b. Salt marsh frequencies over the 30-day period.

a



b

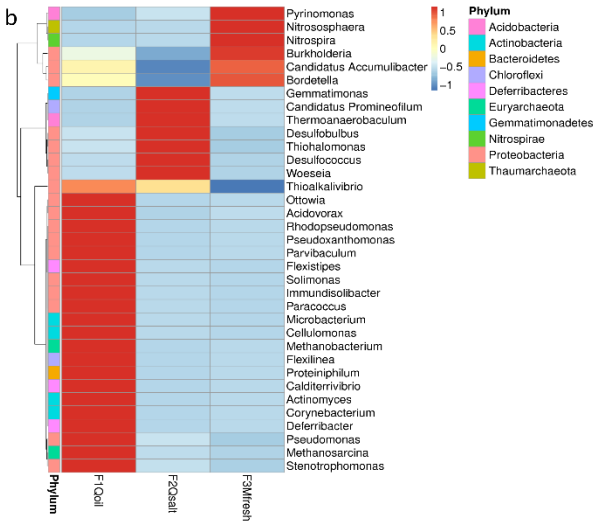


Figure 2. a. X-axis indicates sample name; Y-axis indicates taxonomic information; The clustering tree is at the left side of this chart; The absolute value of "Z" represents the distance between the raw score and the population mean in units of the standard deviation. "Z" is negative when the raw score is less than the mean value, positive when more.

in bacteria makeup between the two samples.

DISCUSSION

The genomic data heat maps show the differences among the two soil samples. This suggests that the soils did have a significant difference in bacterial composition which could be a reason why the two soils performed differently in the MFCs. The two samples had the same amounts of the *Geobacter* genus. The

Bacterial Community

The bacteria domain made up 77% of the freshwater sample. The bacteria community consisted of the following 40% Proteobacteria, 18% Acidobacteria, 14% Nitrospirae, 8% Chloroflexi, 3% Bacteroidetes, 2% Actinobacteria, 2% Cyanobacteria, and the rest were unclassified or less than 1%.

The bacteria domain made up 79% of the salt marsh sample. The bacteria community consisted of the following 42% Proteobacteria, 15% Chloroflexi, 7% Acidobacteria, 5% Gemmatimonadetes, 3% Bacteroidetes, 2% Cyanobacteria, 2% Actinobacteria, 2% Planctomycetes, and the rest were either unclassified or less than 1%.

Within the Proteobacteria phylum in the salt marsh sample the Gammaproteobacteria, 43% of Proteobacteria, and Deltaproteobacteria, 32% of Proteobacteria, classes dominated. Whereas in the freshwater sample the Deltaproteobacteria class made up 32% of the Proteobacteria and the Betaproteobacteria class made up 40% of the Proteobacteria.

With the genomic data of the two samples the heat map shown in figure 2 shows the differences

salt marsh had 0.02% of the *Shewanella*, whereas the freshwater sample had 0.06% Although the *Shewanella* genus was higher in the freshwater samples it did not seem to cause the freshwater to perform significantly better. It could be the reason that the freshwater samples began producing current within the first few days of the experiment where for the salt marsh samples it took more time.

The p-value is 0.01 above the threshold for significance, which could suggest that the there is a

possibility that the salt marsh could produce more power overall. Future studies could possibly do a similar study to see if the salt marsh soils could produce more power over a longer time.

Over the course of the 30 days the salt marsh samples overall produced more power than the freshwater samples, but it was not significantly different. After the 30 days were over the salt marsh samples were still blinking and producing power. The average frequency of blinking from the freshwater samples were 13.02s, whereas the average for the salt marsh were 8.34s. If the length of time had been longer than the 30 days the salt marsh samples most likely would have produced enough power to make the difference significant.

ACKNOWLEDGEMENTS

I would like to thank McPherson College for supporting this research and my education. Thank you to the McPherson College Natural Science Department for advising and funding this research. Thank you to my advisor Dr. Jonathan Frye and Dr. Dustin Wilgers. Also thank you to Dr. Manjula Koralegadara for the encouragement.

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