The incorporation of nitrate in the MFCs tested successfully increased voltage output to some extent. The 10% Coffee Ground Addition MFC gradually began to increase in readings, but quickly decreased. As opposed to the 5% Coffee Ground Addition MFC, in which voltage readings minorly decreased during the beginning of testing but only increased from there on. The 5% Coffee Ground Addition MFC generated the most voltage, when compared to the control and 10% Coffee Ground Addition MFC. The highest voltage reading detected from the control MFC was 542.7 mV, the highest reading detected from the 5% nitrate addition MFC was 669.4 mV, and the highest reading detected from the 10% nitrate addition MFC was 448.7 mV. The 5% nitrate addition MFC increased the maximum voltage output by approximately 23.3462% when compared to the control MFC.

Figure 11, 12, and 13 illustrate the voltage readings across each MFC, with and without the presence of an air pump, at different times throughout the day. All three graphs share the same characteristics, where the 5% coffee ground addition MFC experienced the least fluctuation in voltage readings, the control MFC experienced the most fluctuation, and the 10% coffee ground addition MFC, while it did experience fluctuation in readings to some extent, only majorly decreased in readings approximately halfway through testing. From these results, it can be said that the 5% coffee ground addition MFC had the most balanced supply of nutrients, sourced by the nitrate in the coffee grounds. In addition, nitrate (NO3-) successfully served as a stable terminal electron acceptor in the anaerobic processes involved in the fuel cell. The reason for a rapid increase in readings was since the microorganisms were exposed to a steady nutrient supply, the population of the bacteria increased rapidly. This resulted in the increase of anaerobic respiration, which essentially produced more protons and electrons to be transferred. The more electrons transferred through the external circuit and PEM, the more electricity was produced, and the more water was made in the cathode. In contrast, because the control MFC did not get as stable a supply of nutrients and bacteria was not able to further reproduce to generate more protons and electrons through respiration, the readings fluctuated by a great deal. Observations detected from the 10% coffee ground addition included noting a slimy texture and very stinky odor of the sludge. This is a significant symptom that indicated an unbalance in the C:N ratio (excess of nitrate in this case) is present. Organic matter must have a high enough amount of carbon to give the bacteria enough energy to incorporate nitrogen into their cells. If this does not occur, the nitrogen builds up in the form of ammonia (NH3). The ammonia causes the organic matter to rot and smell bad. When the organic matter rots, the microorganisms in the organic matter began to die, resulting in lower anaerobic respiration levels and lower proton and electron production.

Comparisions between the experimental and control fuel cells were measured by a T-test (two tailed distribution and equal variances between the two groups) using Excel software. The p-value was returned from this test and compared with the alpha (probability of rejecting the null hypothesis when it is true—typically indicates that the data is reliable and non-randomized if the p-value is less than 0.05, or a 95% or higher confidence level). The p-value calculated from the sets of data between the control MFC and 5% coffee ground addition MFC was returned as 4.41169 x 10^-5, as shown by the four asterisks in Figure 15, which is extremely significant and proves the data to be exceptionally reliable. Whereas, the p-value calculated from the sets of data between the control MFC and 10% coffee ground addition MFC was returned as 2.43679 x 10^-3, as shown by the two asterisks in Figure 15, which is significant, but less than the other p-value. This shows that the data between the control and 5% coffee ground addition MFC is the most acceptable non-randomized. Figure 15 also illustrates graphical representations of the variability and chance of uncertainty in the data, indicated by the error bars. All three error bars in the graph are very small, therefore it is shown that the values are concentrated, signaling that the plotted average values are more likely not due to chance.

In earlier research, it was found that aerating the solution inside the cathode chamber of an MFC would increase oxygen levels. Oxygen is utilized as a terminal electron acceptor in the cathode; therefore, an air pump would increase the availability of oxygen electron acceptors. When electrons are transferred to the cathode during redox reactions, the high availability of oxygen electron acceptors make the process quicker, allowing more electrons to flow while the PEM balances charges between the electrodes. However, in this study, although readings of each MFC increased with the air pump, they did not increase by a great deal—only by about 5-20 mV. This could have been changed if the air pump aerated the cathodic solution for a longer period of time, providing more electron acceptors for the incoming electrons.

Although this experiment successfully enhanced the performance of an MFC, several difficulties arose throughout the process. During day 14 of testing, the carbon cloth electrode in the anode of the control MFC broke. The copper wire detached from the carbon fiber cloth, which helped collect the electrons and transfer them to the copper wire. However, when the copper wire was pressed onto the carbon fiber cloth after detachment, readings spiked to 554.7 mV without the presence of an air pump in the cathode, and 569.3 mV with the presence of an air pump. This was most likely due to the force that was applied to push the copper wire onto the carbon fiber cloth, which created a more secure and tighter connection between them. Without the force applied in a regular electrode, only glue was holding the two materials together, resulting in less fixed connection. However, the electrode was immediately exchanged with a spare for the purpose of setting all experimental groups exactly the same, except for the independent variable.

In addition to this setback, no voltage readings were produced when the 220 Ohm resistor was connected to the external circuit, except during the last three days of testing, where the 5% coffee ground addition MFC produced 1.7 mV. This, of course, cannot be used to power anything. Using a derivation of Ohm's Law, P = V^2/R, where P is the power in watts (W), V is the voltage (V), and R is the resistance in ohms (Ω), the power output was calculated and recorded as 0.0016 W on day 19, 0.0017 W on day 20, and 0.0018 W on day 21. From these results, it can be proposed that the 220 Ohm resistor was too strong for this specific study.

Another major obstacle was that the cathode and anode chambers of this experiment were not completely watertight. The chambers were built from cylindrical plastic containers, so the compression fittings (PEM) were not able to be glued flat onto the sides of the container. Thus, the assembly was not 100% watertight. As a result, a few minor leaks were encountered. Although Loctite sealant and Teflon tape were able to stop the leaks, a smarter design choice would be to use flat sided acrylic containers as anodes and cathode. This alternative would allow the compression fittings to be adhered flat onto the sides of the containers, allowing the assembly to be watertight and free from leaks.

Furthermore, the position of the anode and cathode after the compression tubes were screwed on caused an adversity. The holes that were drilled in the anode containers for attaching the compression fittings endcaps were not perfectly lined up to meet the holes drilled in the corresponding cathode containers. Due to this, when the fitting tubes were connected with their endcaps, the chambers were offset in different directions and were not able to stand up straight. However, this issue was solved by filling an aluminum tray with soft dirt, as demonstrated in

Figures 8, 9 and 10. The dirt was used to prop the chambers of the MFCs to stand as straight as possible. This also minimized movement of the MFC because the dirt surrounded the bases of the anode and cathode chamber, stabilizing them. Lastly, the aluminum trays worked conveniently to hold any leaking water.

The final, and perhaps most significant, issue was when the MFCs did not get any readings with the sludge sample from the Ken Malloy Regional Park. When the substrate collected from the Ken Malloy park, it was only tested in one MFC to confirm functionality. However, when testes, no voltage readings were detected. I believe this was either because the anaerobic bacteria died overnight (although they were not exposed to oxygen for longer than 24 hours), or because there were too little microorganisms to produce a significant amount of ATP (protons and electrons) to be transferred and detected. As a result, the Bollona Wetlands and Marsh was visited to sample an alternative sample of sludge. The MFC with the previous (Ken Malloy) sludge sample was dismantled and rinsed, then re-contrsucted to include the Bollona Wetlands and Marsh sludge sample. The MFC successfully produce voltage readings, so the other two MFCs were built just like it. a

To further enhance this study, a bacterial analysis of the organic matter (sludge) can be performed before and after testing nitrates on them. By this, it can be easily observed exactly how nitrates affect anaerobic respiration processes and essentially, how they contribute to the overall performance of an MFC.

The results of this experiment show that a minor addition of nitrate is enough to augment the voltage output of a double chambered microbial fuel cell by an average of 25.7832%, as well as accelerate wastewater treatment. MFCs with an addition of nitrate can be implemented as an eco-friendly and fast energy production and domestic wastewater treatment method in the present-day world. Humanity has only touched the surface of MFC capability. As the understanding of microbial metabolisms deepens, better exoelectrogens can be produced and new applications can be discovered. Eventually, MFCs may take over as the new source of green energy for our Earth.