Manuscript type: Primary Research Article

The Effect of Salt in the Anode Chamber on Voltage Output of a Microbial Fuel Cell

Jeremy Dapaah[1*], Sherrie Feng[1*], Kevin Gu[1*], Emily Ye[1*]

[1] Science and Technology Department, Thomas Jefferson High School for Science and Technology, Arlington, VA, USA

[*]Authors for correspondence:
Jeremy Dapaah, email: 2020jdapaah@tjhsst.edu
Sherrie Feng, email: 2020sfeng@tjhsst.edu
Kevin Gu, email: 2020kgu@tjhsst.edu
Emily Ye, email: 2020eye@tjhsst.edu

Short Title: *Salt in MFC Anodes*

Word Count: 2295

ABSTRACT (210 words)

Energy and waste are two of the biggest problems our society faces. The microbial fuel cell (MFC) has potential as a solution to these problems by being able to simultaneously dilute wastewater and produce electrical current. MFCs consist of two acrylic chambers. In the anaerobic anode chamber of a MFC, bacteria oxidize organic material producing protons and electrons as metabolic byproducts. The freed electrons then travel through an external circuit to the cathode chamber, while protons move to the cathode chamber through the proton exchange membrane (PEM). In the aerobic cathode chamber, electrons react with protons and the newly-available oxygen to form water. The flow of electrons through this circuit generates a current. The purpose of our experiment is to determine whether a presence of a salt in the anode chamber of a MFC would have an effect on the overall voltage production of the cell. We chose to use copper sulfate ($CuSO_4$), an acidic salt; sodium carbonate (Na_2CO_3), an alkali salt; and a control of distilled water with no additional salts. After running a statistical analysis on the average voltages generated by the MFCs, we were able to conclude that the addition of salt to the anode chamber does not have a significant effect on the MFC's voltage production.

INTRODUCTION

Non-renewable energy sources, such as fossil fuels, are not only decreasing in availability, but are also causing disastrous consequences, including global warming and pollution. This has led to a greater focus on renewable energy sources, such as photovoltaics, wind energy, and hydropower. However, the usage of municipal solid waste (MSW), or garbage, to generate energy is often overlooked. Though Americans produced almost 255 million tons of garbage in 2013, only 13% of it was converted to energy ("Waste-to-energy municipal"). Furthermore, according to Cheng and Hu (2010), current methods of converting waste to energy utilize either incineration or gasification, both of which pollute water sources and the atmosphere in the same way as fossil fuels, rendering the advantages of renewable energy sources invalid. The microbial fuel cell (MFC) serves as a solution to this problem, as it harnesses chemical energy from bacteria to convert waste directly into usable electrical energy (Torres, 2012). Moreover, the MFC relies on neither precise environmental conditions nor expensive electrical equipment for efficient operation, further establishing its advantage over other renewable energy sources (Hernández-Fernández et al., 2015). These benefits of the MFC make it practical, even in developing or rural areas. Though the MFC has the potential to make industrial processes more cost-effective, continued improvement of the MFC's efficiency requires additional research (Hernández-Fernández et al., 2015).

The MFC follows a chain of reactions that ultimately turns chemical energy into electrical energy. Similar to a battery, the MFC contains an anode and cathode chamber. A proton exchange membrane (PEM) separates these two chambers (Rahimnejad, Adhami, Darvari, Zirepour, & Oh, 2015). Bacteria in the anaerobic anode chamber oxidize oxygen-deprived organic material, resulting in the production of protons, electrons, and carbon dioxide as metabolic byproducts (Hernández-Fernández et al., 2015). The liberated electrons travel externally through a wire to the cathode, exiting the anode compartment. In contrast, the protons move internally to the cathode chamber through the PEM, and the carbon dioxide diffuses into the air. In the aerobic cathode chamber, electrons react with protons and the newly-available oxygen to form water. The flow of electrons through this circuit generates a current.

In order to generate electricity, the MFC uses microorganisms as a biocatalyst for energy production. The bacterium our experiment utilizes is *Shewanella oneidensis (S. oneidensis)*, which is a nonfermenting facultative anaerobe (Venkateswaran et al., 1999), or an organism that is able to survive in both aerobic and anaerobic environments. According to Biffinger et al. (2008), researchers often use the *Shewanella* family of bacteria in MFCs due to their ability to reduce a variety of terminal electron acceptors. In order to further test the adaptability of *S. oneidensis* for power production, we modulated the salt type in the bacterium environment.

Previous studies suggest that dissolving salt into the anode chamber can have multiple effects on the voltage output of the MFC. For example, table salt (NaCl) is able to improve the voltage production of microorganisms when added in low concentrations (Lefebrve, Tan, Kharkwal, & Ng, 2012). Other salts could potentially affect the MFC's voltage production in a similar manner. Particularly of interest is the potential of common, low-cost salts similar to NaCl. The possibility of increased efficiency through these salts heightens the appeal of the MFC as an inexpensive energy source.

Our experiment tests the conjecture that the presence and type of salt can affect the voltage production of the MFC. However, there is an extensive variety of salts ranging from edible table salts to highly lethal chemical salts. This abundance in salt varieties can be attributed to the dozens of chemical combinations. We chose to use copper sulfate ($CuSO_4$) and sodium carbonate (Na_2CO_3) as our independent variables, with distilled water acting as a control. By choosing an acidic salt like $CuSO_4$ and a basic one like Na_2CO_3, we hoped to account for the effect of pH upon voltage production. We hypothesized that the presence of a salt in a MFC would result in a greater voltage production. Unfortunately, we were unable to confirm this hypothesis, as there was no large difference between the voltage produced by the three cells.

MATERIALS and METHODS

Materials

Before beginning to build our MFCs, we first collected our materials: two acrylic chambers, two rubber gaskets, four bolts, eight washers, four wingnuts, a 3” x 3” proton exchange membrane (PEM), two 2” x 2” carbon fiber pieces (electrode), two 2” x 2” blue J Cloth pieces, two 5” pieces of titanium wire, baby oil, and water. We also gathered the materials necessary to fill the microbial fuel cell. This includes: our salts, $CuSO_4$ (copper sulfate) and Na_2CO_3 (sodium carbonate); our control solution, distilled water; sodium lactate; potassium ferricyanide; and *S. oneidensis*.

Assembly of the microbial fuel cell

The multistep process of constructing our MFCs began with the sterilization of our cells. After carefully removing any dirt contamination within our cells with bleach, we cut the plastic hairs of our electrode pieces, leaving only about a millimeter on each end. We then created a small hook on one end of each titanium wire and wove the wires through the bundles of carbon in the electrode pieces. Next, we inserted the electrode and a blue J Cloth piece into each chamber of our cells. The J Cloth pieces served as a barrier between the electrodes and the PEM.

To complete the build of each individual chamber, we coated the sides of the chambers with baby oil before placing the baby oil-coated rubber gaskets on top.

After finishing the construction of each side of the MFC, we were ready to put the chambers together. First, we removed one of the PEM's protective coatings by tapping the corner with water. This step allows for the PEM's hydrophilic reaction to take effect and allow one of the the protective layers to peel off. Next, we placed the corner of the PEM at the corner of the gasket and removed the second shield using the same water method. Finally, we aligned the holes of the anode and cathode chambers together and punctured holes into the PEM exposed through the holes. This allows for the screws to properly pass through the PEM without disturbing the alignment. Finally to complete the MFC assembly, we pressed the chambers together using screws, washers, wingnuts.

Filling of the microbial fuel cell

To fill our microbial fuel cells, we created 0.1 molar solutions of Na_2CO_3 (5.3 grams) and $CuSO_4$ (7.98 grams), each in 50 mL of water. We then tested the pH of the solutions: 10 for the Na_2CO_3 solution, 6 for the $CuSO_4$ solution, and 8 for the pure *S. oneidensis*. Next, we made the anodic solutions for each MFC. Each one consisted of 2.5mL of the corresponding salt solutions, or water for the control, as well as 22.5 mL of *Shewanella*. We added these solutions to the MFCs with syringes, and filled each cathode chamber with 25 mL of potassium ferricyanide as the catholyte solution. Once the testing started, we added 250µL of sodium lactate to each of the anode chambers as food for the bacteria.

Voltage measurements

To record the voltage generated, we wired the cell to a Labquest and intertwined a 1000 Ohm resistor between the two titanium wires to create a current. We then connected one alligator clip to each wire of the MFC. We set the Labquest to collect a voltage measurement every 108 seconds.

Statistical analysis

We chose to conduct a one-way ANOVA test, with a significance level of 0.05.

RESULTS

We tested the variation and presence of salt type in the anode chamber of an MFC and found that the voltage produced by each MFC varied between trials. Initially, we used a concentration of 0.1 M which resulted in the MFCs producing little to no voltage. Therefore, we changed the concentration to 0.05 M for the remaining trials, which are the focus of our analysis. As shown in Figure 1, the MFC containing Na_2CO_3 produced the most voltage on average, while the MFC containing $CuSO_4$ generated the least average voltage. However, the cell with Na_2CO_3 showed considerably less consistency in voltage production compared to the other two cells, as its standard deviation was five times greater than that of the control and twelve times greater than that of the cell with $CuSO_4$.

We tested the significance of our results using a one-way ANOVA test. The results, as displayed in Table 1, proved that our data are insignificant at the 0.05 alpha level. Therefore, we are unable to reject our null hypothesis, which stated that salt differentiation would not change the amount of voltage produced by an MFC.

We found that the addition of these salts cause the pH to fluctuate due to their acidic or basic nature. We tested the pH of the anode solution before each experiment and found that the MFCs with near neutral pHs tended to produce more voltage than the other MFCs. These results suggest that salt type, concentration, and maintaining a relatively stable, neutral pH are important factors in increasing MFC efficiency.

DISCUSSION

We chose to conduct this experiment in order to observe whether the presence of a salt caused a significant difference in the efficiency of an MFC. We were unable to confirm our hypothesis, which stated that the addition of a salt would increase the MFC's voltage production.

There are many aspects of our experiment that need improvement. For example, the pH of the MFC's anode chamber was a confounding variable that significantly impacted our experiment. Our MFCs produced the most voltage at near-neutral pHs, regardless of the presence or type of salts in the anode chamber. Other studies have shown that pH does have profound impact on MFC voltage production and that neutral pHs do indeed tend to cause higher voltages (Chakraborty, Goyal, Min, & Onteeru, 2017). Therefore, it is necessary to continue testing the effects of various salts on the voltage produced by MFCs, factoring in a greater variety of other possible influences, such as pH.

In addition, there were some inconsistencies regarding the concentrations of each salt in each MFC. Certain errors in our procedures may have influenced our experimental results. For example, the concentration of salt in our first trial was too high, resulting in the death of many of our bacteria and therefore, low voltage production. This made the first trial's results unreliable for analysis.

However, our results did lead us to further investigate our salts' effects on microbes. We found that Na_2CO_3 is an agent for reduction, allowing for quicker electron transport from the anode chamber and thus, higher voltage production (Jin, Huang, Zhang, Jia, & Hu, 2013). On the other hand, $CuSO_4$ is commonly used in pesticides and preservatives, and it can be toxic to microbes such as *S. oneidensis* ("Copper Sulfate," 2017). Therefore, our results in the first trial and even possibly in our later trials could have been severely impacted by $CuSO_4$'s toxicity.

This research is important as it sheds new light on the field of fuel cells. While we were unable to provide significant results, the addition of Na_2CO_3 to the anode chamber did consistently cause higher voltage production. We believe that further research is required to determine if Na_2CO_3 could be a viable addition to the anode chamber for higher efficiency. Conducting studies to further test and determine possible options for increasing bacteria productivity, especially involving Na_2CO_3 and other salts, can lead to improving the overall efficiency of MFCs, thus allowing them to gain recognition as a leading energy source contender.

ACKNOWLEDGEMENTS

This study was heavily supported by Dr. Jennifer James, a former researcher at the National Institutes of Health. She now teaches biology classes at Thomas Jefferson High School for Science and Technology. All the researchers would also like to thank Mrs. Nancy Glover for providing guidance in the creation of this paper, along with Mr. Craig Lewis, who supplied advice on the construction of a microbial fuel cell. They teach English 9 and Design and Technology at Thomas Jefferson, respectively.

REFERENCES

Biffinger, J. C., Pietron, J., Bretschger, O., Nadeau, L. J., Johnson, G. R., Williams, C. C., . . . Ringeisen, B. R. (2008). The influence of acidity on microbial fuel cells containing Shewanella oneidensis. *Biosensors and Bioelectronics*, *24*, 900-905. http://dx.doi.org/10.1016/j.bios.2008.07.034

Chakraborty, N., Goyal, A., Min, D., & Onteeru, M. (2017). pH buffers modulate electrical energy output of microbial fuel cells. *IBET Paper.*

Cheng, H., & Hu, Y. (2010). Municipal solid waste (MSW) as a renewable source of energy: Current and future practices in China. *Bioresource Technology*, *101*, 3816-3824. http://dx.doi.org/10.1016/j.biortech.2010.01.040

Copper Sulfate. (2017, February 4). Retrieved February 8, 2017, from PubChem website: https://pubchem.ncbi.nlm.nih.gov/compound/Copper_Sulfate

Hernández-Fernández, F.J., Pérez de los Rios, A., Salar-García, M.J., Ortiz-Martínez, V.M., Lozano-Blanco, L.J., Godínez, C., . . . Quesada-Medina, J. (2015). Recent progress and perspectives in microbial fuel cells for bioenergy generation and wastewater treatment. *Fuel Processing Technology*, *138*, 284-297. http://dx.doi.org/10.1016/j.fuproc.2015.05.022

Jin, Y., Huang, S., Zhang, M., Jia, M., & Hu, D. (2013). A green and efficient method to produce graphene for electrochemical capacitors from graphene oxide using sodium carbonate as a reducing agent. *Applied Surface Science, 268,* 541-546. https://doi.org/10.1016/j.apsusc.2013.01.004

Lefebvre, O., Tan, Z., Kharkwal, S., & Ng, H. Y. (2012). Effect of increasing anodic NaCl concentration on microbial fuel cell performance. *Bioresource Technology*, *112*, 336-340.

http://dx.doi.org/10.1016/j.biortech.2012.02.048

Rahimnejad, M., Adhami, A., Darvari, S., Zirepour, A., & Oh, S.-E. (2015). Microbial fuel cell as new technology for bioelectricity generation: A review. *Alexandria Engineering Journal*, *54*, 745-756. http://dx.doi.org/10.1016/j.aej.2015.03.031

Sodium Carbonate. (2017, February 4). Retrieved February 8, 2017, from PubChem website: https://pubchem.ncbi.nlm.nih.gov/compound/Sodium_Carbonate

Torres, C. (2012). Improving microbial fuel cells. *Membrane Technology*, *2012*(8), 8-9. http://dx.doi.org/10.1016/S0958-2118(12)70165-9

Venkateswaran, K., Moser, D., Dollhopf, M., Lies, D., Saffarini, D., MacGregor, B., . . . Nealson, K. (n.d.). Polyphasic taxonomy of the genus Shewanella and description of Shewanella oneidensis sp. nov. *International Journal of Systematic and Evolutionary Microbiology*. http://dx.doi.org/10.1099/00207713-49-2-705

Figure 1.

Figure 1. The graph shows that the sodium carbonate MFC produced the highest voltage on average, while the MFC containing copper sulfate produced the least average voltage.

Table 1.

Statistics Summary Table for the Effect of Salt Differentiation on the Voltage Produced by a Microbial Fuel Cell

	Salt in Anode Chamber		
	Sodium Carbonate	Water	Copper Sulfate
Mean Voltage Produced (millivolts)	72.98	46.82	16.37
Standard Deviation	12.5	33.4	9.05
# of trials	2	2	2
One-way ANOVA			0.05 0.14

**p > .05

Table 1. The one-way ANOVA test proved that the results are not statistically significant at a 0.05 alpha level.