Fabrication of Microbial Fuel Cells from Soil-based Electrogenic Microbes and Organic Waste to Generate Bioelectricity

Ng Yan Bin Lucas (3S3), Lucas Koh Eu Jen (3S3), Peng Zi Kang (3S1)

Group 1-43

ABSTRACT:

Microbial Fuel Cells (MFC) is a promising technology and renewable form of energy in the form of biofuels. This study involves the testing of MFCs with potting soil, burnt soil and garden soil, before evaluating the efficacy of lime compost, grapefruit compost as well as strawberry compost in generating electricity. To create the compost, burnt soil was mixed with 500g of each fruit in 3 separate containers and left for 3 months for composting. The set ups were left for one week and the voltage was measured at intervals of every hour. Polarization and power density curves were obtained and the fuel cell's maximum voltage output and maximum power output at various ohmic resistances were determined. Out of the 3 common soils tested, potting soil resulted in the highest voltage and power output. It attained an open circuit voltage of 137mV. Out of the 3 types of compost soils, grapefruit compost had the greatest voltage and power output. It also attained an Open Circuit Voltage of 152 mV. To further evaluate if the efficacy of the MFC could be optimized not just in the anodic chamber containing the soil, aquarium wastewater consisting of nitrate and ammonium ions was used in the cathodic chamber, and it produced greater voltage than the control set-up (burnt soil). In addition, ammonium and nitrate levels in the wastewater dropped drastically. Results of this study provides further information of the use of MFCs to generate electricity and reduce waste simultaneously.

1. INTRODUCTION

Fossil fuels negatively influence the nature owing to the emission of carbon dioxide. Consumption of fossil fuels has severely imperiled human life through its drastic aftermaths, such as global warming and atmospheric pollution (Rahimnejad, Ghoreyshi, Najafpour, Younesi, & Shakeri, 2012). Hence there is a need to explore cleaner alternative sources of energy.

Driven by the increasing concern over the energy-climate crisis and environment pollution, recently, there has been growing interest in double chambered Microbial Fuel Cells (MFCs). According to Imologie, Raji, Gbabo & Okoro (2017), MFC is a bio-electrochemical system that utilises microbes to consume and degrade nutrients to release energy in the form of electrons, protons and carbon dioxide. A MFC (Figure 1) comprises an electron acceptor, cathode and anode, a proton exchange membrane or salt bridge and also the medium with microbial populations (Ucar, Zhang & Angelidaki, 2017). The anodic chamber is connected to the cathodic chamber by a salt bridge, which transfers protons that will meet with the electrons which are transferred by the circuit in which a current is produced (Logan, Min & Cheng, 2005).

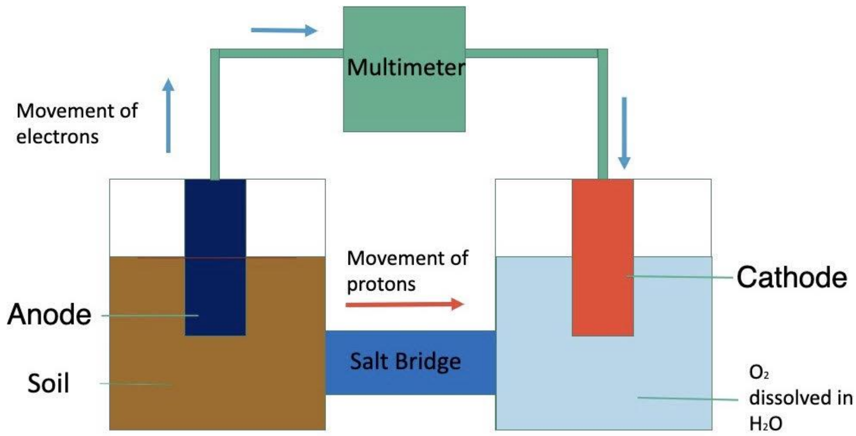

Figure 1: Set up of a Microbial Fuel Cell

Soil is often used as the fuel source in MFC. Soil is rich in microbes like *Deltaproteobacteria*, with 60% of it in the *Geobacteraceae* family which are exoelectrogenic and can produce electricity (Fosso-Kankeu, Marx, Waanders & Jacobs, 2015). An electron acceptor should be efficient in gaining electrons during reduction while the compounds in soil should lose electrons readily, creating a high redox potential for high electron transfer capacities (Ucar *et al.*, 2017).

Microorganisms act as biocatalysts in an analogy to chemical fuel cells. The electrons and protons produced in the anode end up in the cathode via the external electrical circuit for electrons and the exchange membrane for protons. At the cathode, an oxidant (normally oxygen) is being reduced. The equations below illustrate the basic process occurring in MFCs for the case of a glucose-fed system.

Anode: $C_6H_{12}O_6$ (aq) + 6 H_2O (l) → 6 CO_2 (g) + 24 H^+ (aq) + 24e^- (1)

Cathode: 24 H^+ + 24 e^- + 6 O_2 → 12 H_2O (2)

Overall redox reaction: $C_6H_{12}O_6$ (aq) + 6 O_2 (g) → 6 H_2O (l) + 6 CO_2 (g) (3)

This project aims to investigate a more environmentally friendly method to generate electricity. Microbe found in soil mainly came from the *Geobacteraceae* family (Fosso-Kankeu *et al*., 2015), resulting in limitation in diversity of microbial populations, which could be accountable for the low electrical power produced. Thus, this study proposes introducing fruits to enrich soil with more bacteria. Flesh and peels from fruits have been used as substrates in anodic chamber to facilitate direct electron transfer from the fruits. Furthermore, fruits enriches the soil with nutrients like carbohydrates, and the low pH level of the fruit also introduces a new microbial population known as *Acidophiles*, which are exoelectrogens. (Khan & Obaid 2015) However, fruit waste is difficult to obtain in large amounts and quantities. Therefore, creating soil compost to reduce the amount of resources needed while maximizing power output, by introducing both bacteria found in fruits and soil will be more ideal.

Based on several studies, the most common electron acceptor used is oxygen dissolved in water due to its high redox potential, yet it poses challenges due to its poor contact with electrode surface (Ucar *et al.*, 2017). Alternative electron acceptors are thus more promising in increasing voltage and power output. Research has shown that the MFC can remove ammonium and metal ions when electrons and protons reduced them to less toxic forms, serving as alternative electron acceptors(Daalkhaijav, 2012)Wastewater from leachates, brewery and sewages have been utilized but is used entirely in the anodic chamber, which only allows Ammonia Oxidizing Bacteria and Nitride Oxidizing Bacteria to remove harmful pollutants, and reduction of these pollutants are made impossible (Alabiad *et al*., 2017, Ucar *et al.*, 2017). Therefore, this study proposes introducing these bacteria into the aquarium wastewater first, before adding into the cathodic chamber for redox reactions to occur.

There have also been studies on which material are best for electrodes. According to a study conducted by Shen *et al.* (2014), CNFs/GF [Carbon nanofibers with graphite felt] could generate a current density of 3.57 mA cm^{-2}. This shows that CNFs/GF could act as a very suitable cathode. However, CNFs/GF are costly. In this study, graphite electrodes required for MFC were obtained from batteries and used as the electrodes, rendering the MFC fabricated even more eco-friendly.

2. OBJECTIVES AND HYPOTHESES

2.1 OBJECTIVES

1. Construct a double chamber Microbial Fuel Cell (MFC) and evaluate its power efficiency.
2. To investigate the effects of the different types of soil and compost on power output of the MFC.
3. To investigate the effect of the type of Terminal Electron Acceptor (oxygenated water versus aquarium wastewater) on power efficiency and effectiveness in removing ammonium and nitrates.

2.2 HYPOTHESES

1. Potting soil will result in highest power output.
2. Lime compost will result in higher output than strawberry and grapefruit compost.
3. Harmful ammonium and nitrate ions in aquarium wastewater can be removed through redox reactions.

3. MATERIALS AND METHODS

3.1 Materials

Burnt, garden and potting soil were purchased from Corona Florist and Nursery, while potassium chloride and agarose powder were procured from GCE chemicals. Old zinc carbon batteries were obtained from the school's physics laboratory.

Figure 2: Graphite rods extracted from used zinc carbon batteries

3.2 Construction of double-chamber microbial fuel cell

A 3.0cm hole was drilled at the side of each of the 2 containers. 2 small sized connectors were secured within these holes and applied with a thick layer of silicone. Two holes were drilled in one cap for the cathodic chamber and one hole was drilled in the other cap for the anodic chamber. Soil, premixed with 350 ml of 0.28 mol dm $^{-3}$ of glucose solution was added to one side (anode) and water was added to the other (cathode). 1 full tube of agar salt bridge was synthesized by using 40 g distilled water mixed with 10 g of potassium chloride and 1.2 g of agarose powder, and connected tightly to the two ends. 4 graphite electrodes were extracted from batteries, coiled around with thin copper wire and the wires were soldered together to form one set of electrode , which were then applied with parafilm on areas of the electrodes that have copper wire exposed. One set of electrodes are placed into each chamber at a depth of 3 cm with reference to the base and an air pump was connected to the cathodic chamber containing 1L of water. All gaps were sealed and a SparkVue datalogger was connected in series to the set up for 7 days to obtain the voltage obtained. The set-up of the MFC was shown in Figure 3.

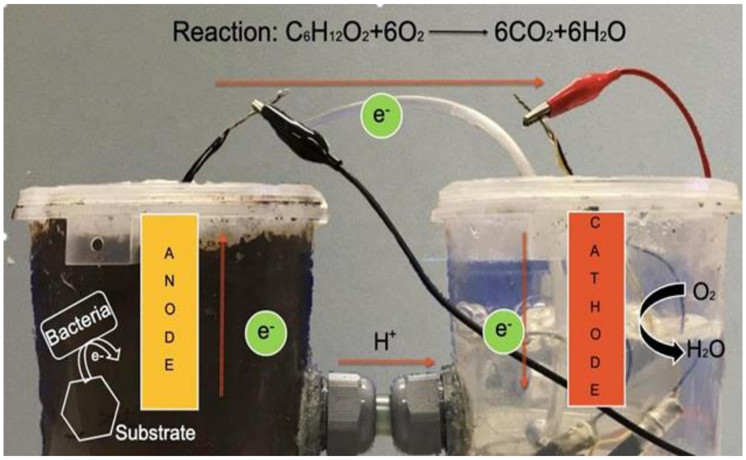

Figure 3: Actual set up of MFC

3.3 Characterization of MFC

Resistors were attached to a circuit board and connected parallel to the MFC. Resistance was varied from 100 to 470000 ohms and the corresponding voltage determined for each type of soil or compost. Current (mA), current density (mA/m^2), power (mW) and power density (mW/m^2) were then computed.

3.4 Evaluating the effect of the type of soil on the voltage generated by the MFC

The different types of soils that were used in this study were burnt soil, garden soil, potting soil, and soil from fruit compost. 1L of soil was poured into a container, and compressed with a mallet. In this study, volume of soil rather than mass was kept constant as different types of soil have different densities and using the same mass of soil would result in the volume being very different. Voltage generated was monitored using a Sparkvue datalogger over 7 days. The maximum voltage generated was determined.

3.5 Removal of ammonium and nitrate from wastewater

1L of aquarium wastewater (from fish tank with 10 guppies (*Poecilia reticulata*) was obtained and mixed with 500 ml of soil and left for 2 hours to allow the essential bacteria to mix with the wastewater containing ammonium ion and nitrate. The suspension was then filtered with a Buchner funnel using vacuum filtration to obtain the filtrate which was placed in the cathodic chamber for redox reactions to occur. The efficiency in removing the pollutant was calculated using the formula below:

Percentage of pollutant removal: $Q_d = \frac{C_b - C_a}{C_b} \times 100\%$

Where Q_d is the percentage of ammonium or nitrate ion removed

C_a is the final concentration of ammonium ion or nitrate /mg L^{-1}

C_b is the initial concentration of ammonium ion or nitrate /mg L^{-1}

4. RESULTS AND DISCUSSIONS

4.1. Characterization of MFC using varied external resistances

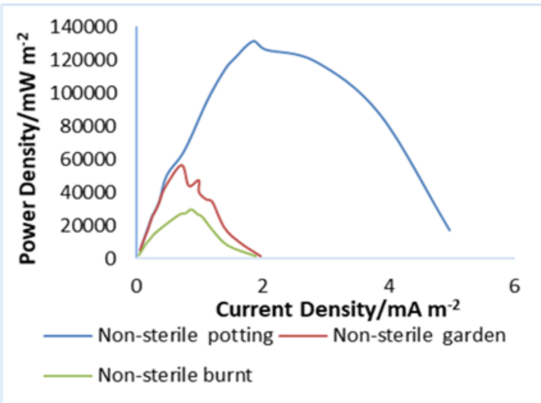

Figure 4 : Power Density curves of the common soils

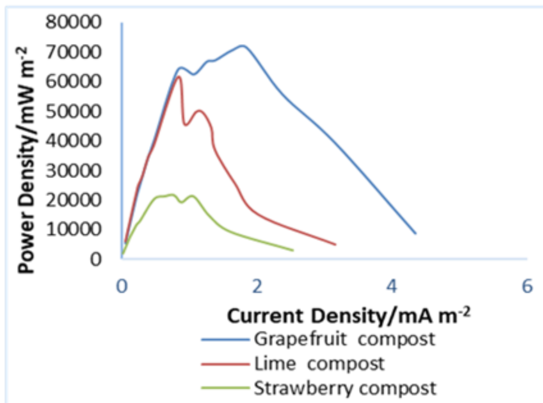

Figure 5 : Power Density curves of the compost soils

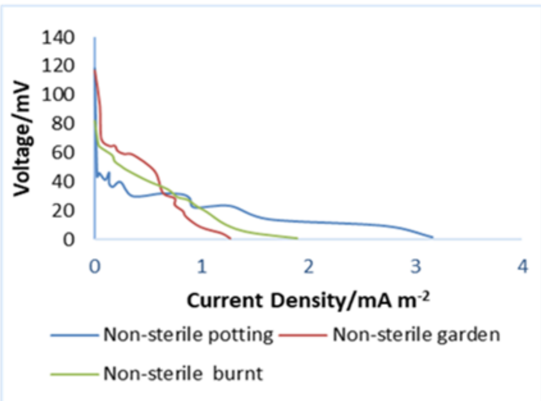

Figure 6 : Polarisation curves of the common soils

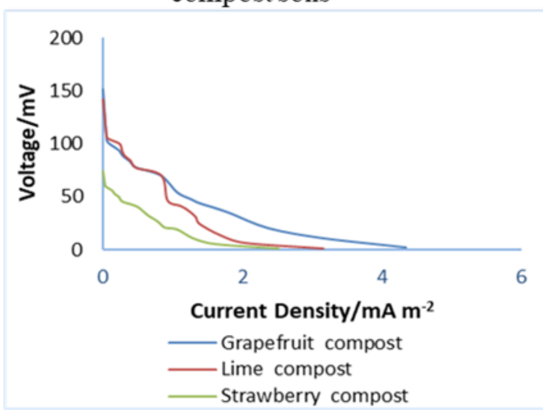

Figure 7 : Polarisation curves of the compost soils

Out of the 3 common soils, non-sterile potting soil shows the most ideal polarization curve (Figure 6) as it has the highest voltage output of 118.3mV, with electrochemical reactions and electron transfer rates being the greatest. Power density (Figure 4) obtained from non-sterile potting soil was also highest at 130W/m^2. Among the 3 types of compost soils, grapefruit has the greatest voltage output (Figure 4) of 151.3mV with power output (Figure 6) of 71.3W/m^2.

4.2 Gram staining of soils

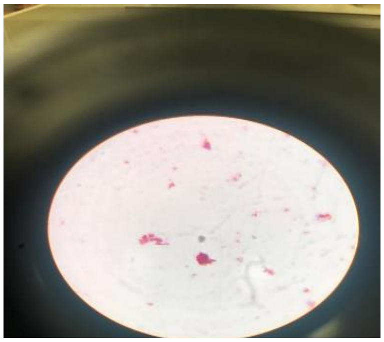

Figure 8: Bacteria in non-sterile burnt soil

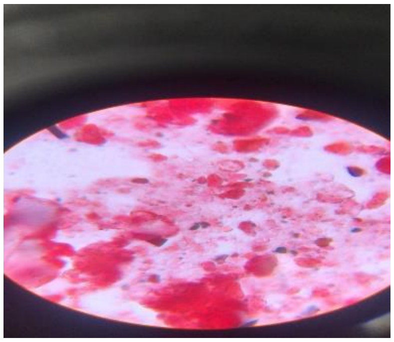

Figure 9: Bacteria in non-sterile garden soil

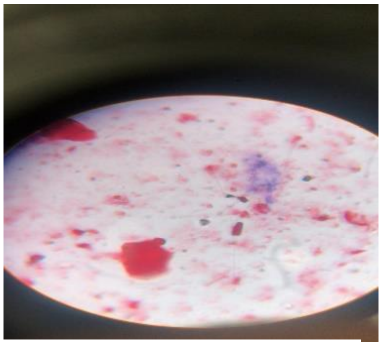

Figure 10: Bacteria in non-sterile potting soil

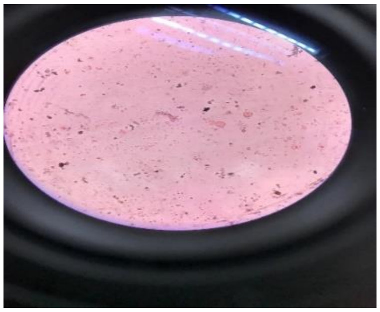

Figure 11: Bacteria in grapefruit compost

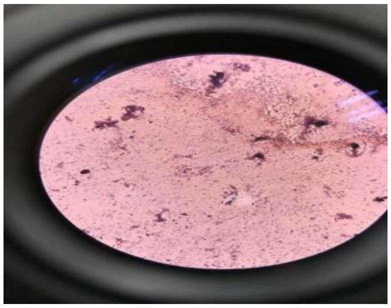

Figure 12: Bacteria in lime compost

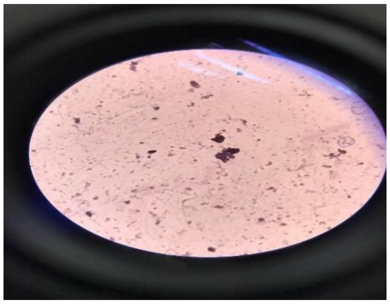

Figure 13: Bacteria in strawberry compost

Gram-positive bacteria were identified based on its unique purple color because of staining of peptidoglycan cell wall while red stains from safranin are characteristic of gram-negative bacteria. Non-sterile potting soil (Figure 10) and non-sterile burnt soil (Figure 8) show communities of gram-negative bacteria while grapefruit compost (Figure 11) shows both gram-negative bacteria and gram-positive bacteria. In contrast, lime compost and strawberry compost contain gram-positive bacteria. Gram-negative bacteria rely on cell surface exposed cytochromes for the oxidation or reduction of minerals and organic material extracellularly. Furthermore, potting soil contains other minerals, like calcium and iron, where gram negative bacteria can thrive because the mineral-respiring gram-negative bacteria works by redox-active molecules in their outer membrane to transfer electrons by relying on cell surface exposed cytochromes for the oxidation or reduction of minerals and organic material extracellularly (Hernandez & Newman, 2001). This explains why potting soil has high voltage output which is similar to grapefruit compost (Figure 11), which produced highest voltage out of the 3 compost soils.

4.3 Voltage peaks of soils over 7-day period

Potting soil achieved the highest voltage peak which shows that it is a good medium for the growth of bacteria. The voltage peak represents the non-constant maximum voltage output of the MFC. P-value of Kruskal Wallis Test is determined to be 0.033, indicating that there is a significant difference in voltage peaks generated by these common soils. When using Mann Whitney U Test to compare burnt soil against potting soil and burnt soil against garden soil, the former pair yielded a p-value of 0.037 while the latter had a p-value of 0.173, highlighting that the voltage peak of potting soil is significantly different from burnt soil but that of garden soil is not.

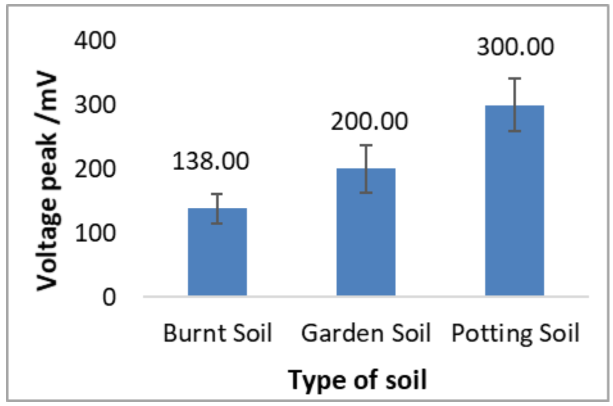

Figure 14: Voltage peaks of the various common soils from overtime testing

4.4 SEM images of electrodes

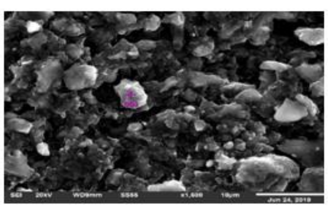
Figure 15: SEM image of control electrode

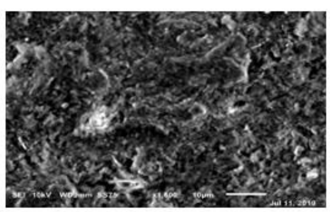
Figure 16: SEM image of electrode in burnt soil

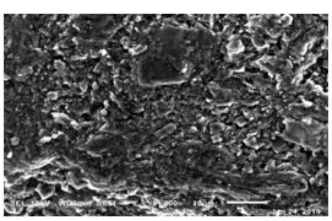
Figure 17: SEM image of electrode in garden soil

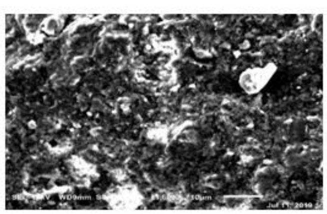
Figure 18: SEM image of electrode in potting soil

SEM images of burnt, garden and potting soil electrode (Figures 16, 17 and 18) reveal a much smoother surface as compared to the surface of the control, which has a rougher surface with discrete particles being observed. Potting soil has the greatest amount of biofilm. Biofilm that formed over the graphite electrodes of the soil increases the voltage of the MFC. The biofilm comprises mainly gram-negative bacteria, which are more adaptable than gram positive bacteria in the soil conditions (Arbianti *et al.*, 2018), therefore they are able to reproduce more rapidly as compared to gram positive bacteria. Furthermore, bacteria like *Geobacter sulfurreducens,* which forms a biofilm on the electrode, shows best extracellular electron transfer (EET) performance as it can undergo direct electron transfer due to its multiheme cytochromes (Zhou & He, 2009). Thus potting soil has the highest voltage.

4.5 Voltage peak of aquarium wastewater

As shown from Figure 19, the wastewater set-up had a higher peak voltage than the control set-up, but the difference is not significant as the p-value of Mann-Whitney U Test is 0.169, which is greater than 0.05. However, it highlights that ammonium and nitrates are suitable alternatives to oxygen as electron acceptor.

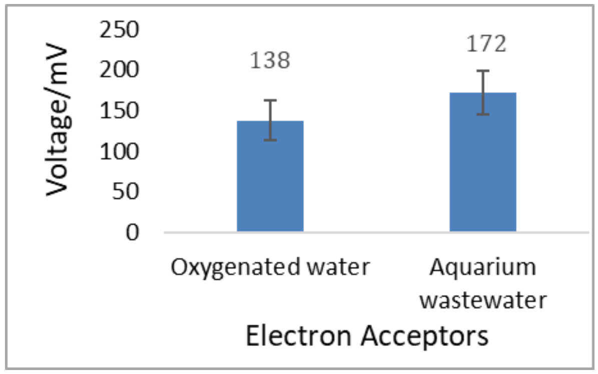

Figure 19: Type of terminal electron acceptor against voltage produced

4.6 Efficiencies of ammonium and nitrate removal

As shown from Figure 20, 71.85% of ammonium ions and 81.06% of nitrate ions had been removed when aquarium wastewater is used as the cathode chamber. This is due to nitrification and denitrification by the Ammonia Oxidising Bacteria (AOB) and Nitrite Oxidising Bacteria (NOB). Ammonium ions could have also been removal by the soil as organic matter in soil contain negatively charged silicates, carboxyl and phenolic groups (Jaremko & Kalembasa, 2014) which attracts positively charged ammonium ions.

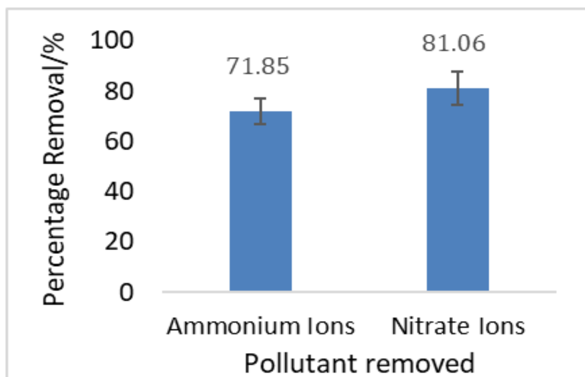

Figure 20: Removal of ammonium and nitrate ion by MFC

5. CONCLUSION AND FUTURE WORK

Potting soil is the best soil among the 3 common soils that have been tested, producing the highest maximum voltage and power output and is hence recommended to be used in the compost making process. Grapefruit compost has the highest maximum voltage and power output compared to lime and strawberry compost and is hence recommended to be used in the fruit composts while using potting soil as medium due to the presence of both gram-positive and gram-negative bacteria, to attain the highest voltage. Wastewater treatment was found to be feasible with MFC technology, greatly reducing the amount of ammonium and nitrate ions in aquarium wastewater. The use of wastewater containing ammonium and nitrate ions was determined to be a suitable replacement for the conventional electron acceptors such as oxygen, producing higher voltages. Compared to conventional technologies of producing energy which utilizes fossil fuel, MFC is greener alternative. MFCs have great potential to be used in widespread applications such as electricity generation and wastewater treatment.

Some future work that can be carried out is the investigation of other types of soils and other types of fruits for the fruit composts in terms of voltage output. Enhancing the electrodes with manganese dioxide can also be explored so as to improve the efficiency of the electrodes. Finally, MFCs can be connected in series and due to its size, it could be placed in the garden, with soil as the medium to grow plants and at the same time generate electricity for lighting up LED lights and plant-watering sensors (Figure 21).

Figure 21: Application of MFC in garden to power LED lights and plant-watering sensors

MFC could also be used by households very conveniently as materials required to fabricate the MFC can be procured from convenience stores or through recycling. For instance, waste fruits peels and flesh could be thrown into soil in the home garden to form compost to generate even more voltage. Aquarium tank water could also be purified by the MFCs while at the same time electricity can be generated to power small devices.

REFERENCES

Alabiad, I., Ali ,U F M., Zakarya, I A., Ibrahim,N., Radzi, R W., Zulkurnai ,N Z.,and Azmi, N H.(2017) Ammonia removal via microbial fuel cell (MFC) dynamic reactor . *IOP Conf. Series: Materials Science and Engineering 206*.
https://iopscience.iop.org/article/10.1088/1757-899X/206/1/012079/pdf

Arbianti, R., Utami, T. S., Leondo, V., Elisabeth, Putri, S. A., & Hermansyah, H. (2018). Effect of biofilm and selective mixed culture on microbial fuel cell for the treatment of tempeh industrial wastewater. *IOP Conference Series: Materials Science and Engineering*, *316*, 012073. DOI:10.1088/1757-899x/316/1/012073.

Daalkhaijav, U. , (2012) Removal of Ammonia (nitrification) in conventional and Microbial Fuel Cell bioreactors. Sourced from
https://pdfs.semanticscholar.org/628d/cc802145f4dda8ad9e1a439ad5a712205963.pdf

Fosso-Kankeu, E., Marx, S., & Waanders, F., Jacobs, V. (2015). Impact of soil type on electricity generation from a Microbial Fuel Cell. *7th International Conference on latest Trends in Engineering and Technology*, 978-93-84422-58-2 ,73-77.
https://www.researchgate.net/publication/286018986_Impact_of_soil_type_on_electricity_generation_from_a_Microbial_Fuel_Cell

Hernandez, M. ,Newman, D. (2001) Extracellular electron transfer.*CMLS. Cell. Mol. Life Sci*., 58: 1562
https://www.ncbi.nlm.nih.gov/pubmed/11706984

Imologie,S.,Raji,O.A., Gbabo, A. & Okoro, S. (2017). Performance of a Single Chamber Soil Microbial Fuel Cell at Varied External Resistances for Electric Power Generation. *Journal of Renewable Energy and Environment, 3,* .53-58.
https://www.researchgate.net/profile/Simeon_Imologie/post/How_to_calculate_power_density_and_current_density/attachment/5a2710d54cde266d58788e81/AS%3A568320832098309%401512509653656/download/Jree+MFC+Published+article.pdf

Jaremko, D., Kalembasa, D. (2014). A Comparison of Methods for the Determination of Cation Exchange Capacity of Soils. *Ecol Chem Eng S., 21(3)*, 487-498.
DOI: 10.2478/eces-2014-0036

Khan, A M., & Obaid, M. (2015). Comparative bioelectricity generation from waste citrus fruit using a galvanic cell, fuel cell and microbial fuel cell. *Journal of Energy in Southern Africa*, *26*(3), 90-99.
https://journals.assaf.org.za/index.php/jesa/article/download/2143/1964

Logan, B.E., Cheng,S., Min, B.(2005). Electricity generation using membrane and salt bridge microbial fuel cells .*Water Research, 39,* 1675-1686.
https://www.engr.psu.edu/ce/enve/logan/publications/2005-Min-etal-WatRes.pdf

Rahimnejad, M., Ghoreyshi, A., Najafpour, G., Younesi, H., Shakeri, M. (2012). A novel microbial fuel cell stack for continuous production of clean energy
Int. J. Hydrogen Energy, *37*, 5992-6000.
https://www.sciencedirect.com/science/article/pii/S0360319911028886

Shen, Y., Zhou, Y., Chen, S., Yang, F., Zheng, S., & Hou, H. (2014). Carbon Nanofibers Modified Graphite Felt for High Performance Anode in High Substrate Concentration Microbial Fuel Cells. *The Scientific World Journal, 2014,* 1-5. doi:10.1155/2014/130185.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4030554/

Ucar, D., Zhang, Y., & Angelidaki, I. (2017). An overview of electron acceptors in microbial fuel cells. *Frontiers in Microbiology*, *8*, 643.
https://www.frontiersin.org/articles/10.3389/fmicb.2017.00643/full

Zhou, J., & He, Z. (2009). The Shewanella Federation: Functional Genomic Investigations of Dissimilatory Metal-Reducing Shewanella.
DOI:10.2172/946694