Microbes and Mealworms

Generating Energy Through Plastic Bio-depolymerization

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SCI 400: Research Project

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Abstract

We sought to address two issues within the scope of fossil fuel reduction in this project: alternative sources of energy and plastic bio-depolymerization. Our goal was to create a solution that synthesized these issues by constructing a microbial fuel cell (MFC) that generated energy from plastic-fed mealworm frass (PFMF). We tested the PFMF MFC's voltage production against a positive control condition that was fed sucrose, and a negative control condition that was unfed. We hypothesized that the PFMF MFC condition would generate relatively low voltage and predicted that the voltage output of the conditions from highest to lowest would be positive control, then experimental, and then negative control. We found this to generally be the case but did not generate sufficient data for statistically significant results. The averaged mean voltage values for each condition were as follows: 9.5 ± 1.4 mV for the positive control, 7.2 ± 1.0 mV for experimental, and 7.9 ± 3.6 mV for the negative control. Despite the lack of statistical significance between the conditions' mean voltage output, we were able to accomplish our goal of generating low voltage in the PFMF MFC condition. This indicates that our system is functional and is viable for further exploration and optimization.

Introduction and Literature Review

Alternate sources of energy to fossil fuels are in increasingly high demand as the use of petroleum-based products drive the global crises of pollution and climate change. The purpose of this project is to address two problems within this scope: alternate sources of energy with increased sustainability and circularity, and fast, affordable plastic depolymerization. Solutions to these issues are typically separate and often hard to come by. Many sustainable energy sources pose significant financial and practical barriers on implementation (Unuofin et al. 2023), and much of the plastic produced each year ends up in landfills (Yang Y. et al. 2015), where its degradation results in the introduction of microplastics to the environment.

However, recent research has indicated that microbiologically derived energy could be optimized into a circular, more sustainable small-scale alternative to fossil fuel combustion, and that plastic biodegradation can be performed by macroinvertebrates. Previous research has documented and tested these concepts separately, but not in conjunction. We sought to synthesize these concepts and construct a microbial fuel cell (MFC) that feeds on the plastic-fed mealworm frass (PFMF), creating a circular system that generates electrical energy from biologically depolymerized plastic.

Microbial Fuel Cells

As described by Robinson in their 2017 article, a MFC derives electrical energy from the metabolic activities of exoenergic, typically anaerobic microbes, which can feed on an incredible diversity of substrate in a multitude of environments. Robinson details steps to construct a MFC structurally similar to a simple voltaic cell, with microbial substrate in the anodic chamber connected via a salt bridge to the cathodic chamber that contains saline solution. The electrodes can be made of a variety of materials; Robinson utilized folded pieces of aluminum mesh. Bacteria in the anodic chamber form a biofilm on the surface of the anode and, under anerobic conditions, transfer electrons directly to the

anode. By varying conditions in the MFC, the electrical output of the cell can be optimized to increase voltage and current, and decrease resistance, thus generating more power.

Unuofin and associates explored optimization strategies and provided a broad profile of MFCs in their 2023 review article. They found that while MFCs show promise as sustainable energy sources, there are many challenges to overcome for successful implementation. Because of the wide range of styles and applications of MFCs, electrical output can vary drastically not only between different systems, but within an individual system. This is due to a multitude of factors but is primarily centered on the MFC's microbial community and the substance(s) it consumes. Microbial substrate for MFCs is often gathered from natural sources such as wetlands, as will be the case for this project, resulting in an uncontrolled community that may not all exhibit exoenergic properties or be capable of biofilm formation. Non-exoenergic microbes can compete with exoenergic microbes and limit the MFC's potential, both by consuming resources and occupying biofilm space on the anode. Similarly, if the feeding substrate contains limited or inconsistent nutrients, exoenergic microbes will not be capable of producing a consistent supply of electrons. This is particularly relevant in the scope of this project, as the experimental feeding substrate, plastic-fed mealworm frass, is likely to be less nutrient-dense than the organic matter the microbes normally feed on. However, as will be detailed later, frass from plastic-fed mealworms may also contain other sources of nutrition such as shed cuticles that would help to increase the nutritional value of the frass (Yang Y. et al. 2015).

MFCs are typically either dual- or single-chambered, with each posing separate challenges for optimization. We will employ a dual-chambered system in this study, eliminating the need to account for electrode spacing and ease of access to the anodic chamber, however, as Unuofin et al. discuss, this system does limit oxygen access to the cathode. One of the methods they propose to account for this limitation is the addition of photosynthetic microbes (typically algae) in the cathodic chamber to generate oxygen. An electrically powered aeration device would also mediate limited oxygen but would

require external energy input to the system. As suggested by Dr. Mark Vaughan, a renewable energy source (solar, wind, etc.) should be used to power the aerator to avoid confounding the goal of creating a sustainable, circular system. In this regard, electrode material must also be considered. Unuofin and associates emphasize the importance of electrode material both in minimizing resistance in the cell and allowing for biofilm production on the anode. Metals such as platinum are often used for their high conductivity and low internal resistance but are unideal due to the unsustainability of mining and metals' lack of renewability. Thus, Unuofin et al. suggest that composite electrodes from other organic wastes are preferable. In this study, we will create carbonized electrodes from old cloth encased in recycled metal mesh to facilitate anodic biofilm formation and maintain low internal resistance.

Our electrode design decision was informed by Dr. Vaughan and the results of a 2022 study by Simeon and associates. Researchers compared MFC voltage potential among several variables within a single-chambered soil MFC and found that the system reached maximum sustainable potential with stainless steel-epoxy-carbon black composite (SEC) electrodes positioned 4 cm apart when it was fed every 8 days. The power generated in these conditions was consistently between 0.40 and 0.65 mW for approximately 50 days. The comparative electrode material was carbon felt, which exhibited significantly higher internal resistance and lower surface area and thus limited the power output of the MFC. The design of our electrode is roughly modeled after the SEC electrode. However, the validity of the argument that SEC outperforms carbon felt as an electrode is limited by the team's study design. Simeon et al. assert that the SEC had a larger surface area and lower internal resistance than the carbon felt, but that the carbon felt had higher conductivity, and therefore, conductivity was less relevant to electrode efficacy than resistance. However, this statement is not directly supported by the data, as they did not test the effects of equalizing the surface area of the electrode types. It is thus not certain that internal resistance alone outweighs the effect of conductivity if surface area remains unstandardized between the treatments. While out of the scope of this project, surface area augmentation of various electrode

materials would likely be highly beneficial in the optimization of MFCs and could be tested in our system as an extension of this project.

Biodegradation of Plastics

Multiple species within the *Tenebrio* genus have shown the ability to consume and survive with only plastic as a food source, with a particular emphasis on *Tenebrio molitor* larvae, known as yellow mealworms. In their two-part 2015 study, Yang Yu and associates sought to test the efficacy of polystyrene (PS) biodegradation and mineralization by yellow mealworms and yielded very promising results. Three groups of mealworms were purchased (Beijing and Hebei, China, and Ham Lake, Minnesota) to be used both in control (bran-fed) and treatment (PS-fed) conditions. They found that mealworms could consume PS as their sole food source with little to no effect on survival rates as compared to control mealworms, and that individuals in both treatments hatched into beetles at the end of the testing period. However, the bran-fed individuals gained substantially more biomass over the course of the experiment than the PS-fed group (>30%), signifying that while the PS-fed group survived, they were likely not as well-nourished as the bran-fed group. These findings do not have any negative implications on the scope of this study, however, if this model was to be applied on a larger, more circular scale, issues may occur. An ideal mealworm frass fed MFC system would be self-sustaining, with mealworms hatching into beetles which then would lay eggs to hatch into more mealworms. If PS is not a sufficient food source on its own, the reproductive capacity of the beetles would likely be affected over time, reducing the overall circularity of system as mealworms would have to be purchased and added to the system.

Yang Yu and associates corroborated biodegradation of PS by mealworms by characterizing the PS-fed group's frass via gel permeation chromatography (GPC), solid state 13C cross-polarization/magic angle spinning NMR (CP/MAS NMR), and thermogravimetric Fourier transform IR (TG-FTIR). Spectra

indicated that styrene, the primary product of PS depolymerization, was present in very small concentrations while smaller products such as carbon monoxide and carbon dioxide were prevalent. This indicates that mealworms were able to depolymerize PS beyond styrene, which bodes well for the frass' capability to be utilized as a food source for microbes that may not have the capacity to efficiently degrade larger synthetic compounds. They also assessed carbon dioxide mineralization via 13C-flagged polystyrene and found that less than 1% of PS carbon was absorbed by the mealworms, with the majority used in lipid metabolism. A temporal assessment also revealed that as the study progressed, PS-fed mealworms degraded and mineralized PS with increasing efficiency, with egested and mineralized 13C decreasing and increasing by 50%, respectively, over the 16-day trial. While this is not conclusive evidence that mealworm biodegradation of PS will produce usable products for a MFC, Yang Yu and associates' spectral data also identified peaks in PS-fed mealworm frass associated with shed cuticles. The extra nutrition derived from ingesting these chitinous skins will thus likely extend into the frass and provide more resources for MFC consumption than just present in the biodegraded PS, increasing the viability of our proposed system.

Further investigation into the mechanisms of biodegradation by the mealworms necessitated a second part to Yang Yu and associates' study, wherein the role of the mealworm gut microbiome was assessed. Antimicrobials were administered to PS-fed mealworms to determine if gut microbes were responsible for PS biodegradation. Of the six antimicrobials tested, gentamicin yielded the most drastic results. Characterization of frass and 13C-flagging indicated that individuals fed gentamicin were significantly less able to digest and mineralize PS than mealworms who were not fed gentamicin, validating the researchers' hypothesis that the gut microbes are responsible for the biodegradation of PS, assisted by mechanical digestion performed by the mealworms. The team then cultured gut microbes from PS-fed mealworms to assess community composition and found an abundance of Exiguobacterium sp. strain YT2. These bacteria were able to form a biofilm on and degrade PS surfaces.

Interestingly, one of the main degradation methods of the bacteria was to increase hydrophilicity on the PS surface, which was hypothesized to allow for greater biofilm development and subsequent biodegradation. The degradation products were also hydrophilic, though produced with reduced efficiency compared to in vivo conditions. This implies that mechanical digestion by the mealworms, and interactions with other microbes and endogenous compounds in the mealworm gut aid in biodegradation performed by Exiguobacterium sp. strain YT2. However, Yang Yu and associates only used one source of mealworms in this study (Beijing) in contrast to the three sources used in their previous study. This limitation introduces several barriers in interpreting and applying their results, as mealworms from different locations reared on different food stocks likely have variant gut microbiomes that would perform differently both in vitro and in vivo conditions. Hence, it is challenging to predict if the mealworms purchased for this study will yield similar PS biodegradation products, and consequently, if the MFC will be capable of metabolizing these products.

Nevertheless, other studies have yielded complementary results to Yang Yu and associates' findings while employing different sources of mealworms. Yang Shan-Shan and associates in a 2018 study compared biodegradation of seven types of PS by mealworms purchased in California to corroborate the effects of environmental factors, including the addition of bran to their diet, on the rate of biodegradation and reproductive capacity. They found, through similar methods of frass characterization (GPC, 1HNMR, and TG-FTIR), that mealworms were capable not only of depolymerizing PS, but also of modifying the products, primarily via the cleavage of aromatic rings and the addition of carbonyl groups. This result also supports Yang Yu and associates' findings that mealworm gut microbes biodegraded PS into more hydrophilic products and indicates that PS biodegradation by mealworms will produce compounds that are likely more bioavailable to microbes in a MFC than products of typical PS depolymerization, such as styrene, due to membrane solubility. Yang Shan-Shan and associates also found that PS biodegradation by mealworms became more efficient over the course of the study, but

noted a plateau was reached after approximately 20 days. The mealworms that ate only PS in Yang Shan-Shan and associates' study hatched into beetles, but the beetles were not able to reproduce. Conversely, the beetles that ate PS and bran were able to reproduce, and the second generation showed a slightly increase in PS degradation efficiency compared to their parents. The researchers suggest that mealworms could be selectively bred for PS biodegradation capabilities; we hypothesize that isolating PS-biodegrading microbes from the guts of highly efficient PS-degrading mealworms and introducing these microbes to other colonies of mealworms would have a similarly positive effect on PS biodegradation.

He and associates likewise found in their 2023 study of polyester resin biodegradation by mealworms that the mealworms became more efficient at degrading the microplastic residues over the course of the trials but that a plastic-only diet was likely not sustainable in the long run. Because the goal of this project is to test the efficacy of a plastic-only diet for the mealworms and MFC, this temporally limiting factor will not be addressed. However, further research could be conducted to observe the effects of adding other food sources into the mealworms' diet to supplement nutrition, as demonstrated by Yang S.S. et al. (2018). Additionally, the contribution of organic substrate to the mealworm diet could have a positive effect on MFC performance in our proposed system, as microbes in the fuel cell would likely benefit from more diverse, nutritionally dense food.

Synthesis

Evidence from the literature supports the basic functionality of our proposed system, wherein the larvae of *T. molitor*, yellow mealworms, are fed plastics to biodegrade and egest as frass, which is then fed to a MFC to generate electrical energy. There is significant evidence that mealworms can successfully biodegrade plastics into small, relatively water-soluble products (Yang Y. et al., 2015 and Yang S.-S. et al., 2018) and that MFCs are able to produce electrical energy from a wide range of feeding

substrates (Unuofin et al., 2023). However, because of the high variance of microbes within naturally sourced microbial substrates and the gut microbiomes of mealworms, it is challenging to predict whether the PFMF will be a suitable feeding substrate for the MFCs. If the mealworms obtained are capable of biodegrading plastics in a manner consistent with the literature, and the microbial substrate for the MFCs contain an adaptable enough community of exoenergic microbes, we hypothesize that the proposed system will produce a relatively low voltage potential that can be optimized in subsequent studies. The null hypothesis we will seek to assess is that there will not be a statistically significant difference between the voltage produced by the experimental and control conditions, and conversely, our alternative hypothesis will be that there is a significant difference. To test this hypothesis, we will create three treatment conditions with five MFCs in each. First, the positive control, which will be fed sucrose, serve to control for microbial substrate quality, and represent a normally functioning MFC. Second, the experimental condition, which will be fed PFMF, and serve as the treatment group. Third, the negative control, which will be unfed, serve to control for low voltage output of the cells, and represent a non-functioning MFC. The experimental condition can thus be compared to a condition that we expect to have a voltage output and one that we expect to have a low or nonexistent voltage output. We therefore predict that the average voltage for the conditions from highest to lowest will be positive control, then experimental, and finally negative control.

Methods

Microbes

Microbial Fuel Cell Set-Up

To assess the efficacy of the plastic-frass-fed microbial fuel cell (PFMF MFC), we created fifteen microbial fuel cells per Robinson's (2017) guidelines with modifications. Salt bridges were created using 10 cm lengths of ½ inch flexible plastic tubing, which were inserted directly into the ½ inch holes drilled

in the anodic and cathodic chambers. We then sealed around the tubing with silicone caulking and allowed the seals to set overnight. Distilled water served as the cathodic substrate rather than saline solution.

We obtained soil from a site adjacent to a wetland on Capilano University's North Vancouver campus (see Supplementary Information). The site was chosen as it was clear of plants and surrounded exclusively by invasive Himalayan blackberries to avoid damaging any living plants, particularly native species. The surface layer of leafy debris and dry top layer of soil were cleared to access damp, black soil 2 to 20 cm beneath the surface. We filtered as much debris (sticks, rocks, etc.) out of the substrate as possible during collection. We added approximately 200 mL of microbial substrate to each anodic chamber while further filtering for debris. The microbial substrate was mixed and compacted in the chambers to eliminate as many air pockets as possible and optimize anerobic conditions.

We constructed 30 electrodes per *Autodesk Instructables* user drdan152's (2013) guidelines. 30 pieces of char cloth were created by placing two approximately 7.5 x 9.5 cm pieces of cotton cloth in a metal tin with a hole in the lid over a Bunsen burner and heating until the fabric was completely blackened. We folded the pieces of char cloth in half lengthwise and wrapped each in an approximately 10 x 9.5 cm piece of stainless-steel mesh to generate an electrode approximately 8 x 3 x 0.5 cm in size. These electrodes were employed in both the anodic and cathodic chambers of the MFCs. We connected long wires to the electrodes with alligator clips and submerged as much of the anode in the microbial substrate as possible (approximately 90-95% submerged). The fifteen prepared MFCs were then left unaltered for a 24-day acclimation period to facilitate biofilm formation on the anode.

We then added 200 mL of distilled water to the cathodic chambers and submerged the cathodes. Five MFCs were randomly assigned to each of the three conditions: positive control/sucrose-fed, experimental/PFMF-fed, and negative control/unfed.

Voltage Monitoring

To monitor the voltage of the MFCs during the experimental run, we assembled a circuit containing an Arduino MEGA board, a MicroSD card adapter with a 16 GB MicroSD card, a TCA9548A I2C multiplexer, a 128 x 32-pixel OLED display, and a DS3231 real-time clock (RTC) for each condition. This resulted in three circuits, each monitoring five MFCs, which were assembled as follows:

We connected the MicroSD card adapter directly to the Arduino MEGA per Schoeffler's (2017) schematic and inserted the 16 GB MicroSD card into the port. We then connected the I2C multiplexer to the Arduino MEGA per Adafruit user lady ada's (2015) schematic. The OLED display was connected to the I2C multiplexer channel 0, and the RTC was connected to channel 5. Each of the five MFCs in each condition was connected to a pair of analog pins on the Arduino board, with the cathodic lead at the lower pin and the anodic lead at the higher pin; for example, the cell 1 was connected at the cathode to A0, and at the anode to A1, cell 2 to A2 and A3, etc. A link to the source code for these circuits can be found in the Supplementary Information section of this report.

Feeding

We fed the MFCs three times over the course of the experimental run; on November 10, 17, and 24. Each cell in the positive control condition received 2.00 ± 0.01 g of sucrose, and each cell in the experimental condition received the same amount of PFMF. We stirred the microbial substrate in all conditions after feeding, including the negative control condition, to account for the effects of aeration. We also added 10 mL of distilled water to all cells in all conditions during the second feeding (Nov. 17).

Mealworms

We generated feed for the PFMF-fed experimental condition by constructing a frass-collecting mealworm habitat and obtaining used plastic to feed the mealworms. The habitat consisted of two nesting 5.8 L rectangular plastic containers. We removed the bottom of one container and replaced it

with fine stainless-steel mesh, which was secured in place with aluminum foil tape. Holes were drilled in the lid of this modified container for ventilation. The modified container was nested inside the unmodified container to create a habitat that allowed frass to collect in the lower container.

We purchased two batches of 4000 mealworms from Wild Birds Unlimited in Surrey, BC, first in early October, then early November. We used a fine mesh sieve to separate the mealworms from their original feeding substrate and added them to the mealworm habitat. Two PS meat trays were washed and cut into small (approximately 5 x 5 cm) pieces and added to the habitat. Five expanded polyethylene (EPE) fruit nets were cut into small strips and added to the habitat. We stored the mealworms in their habitat in a fume hood in the biology department of Capilano University's North Vancouver campus.

Frass was obtained every 6-14 days by lifting the modified upper container out of the lower unmodified container and pouring the collected frass into a separate tared container. The mass of PFMF was recorded after collection. We sealed the PFMF container with plastic wrap and a tight lid and stored it in the refrigerator at 4°C.

We observed in the first few weeks of feeding that activity was generally low among the mealworms. Many individuals were not feeding, and movement was limited. The literature suggested that mealworm performance was likely to improve at temperatures between 25 and 30°C (Yang S.-S. et al., 2018 and Deruytter & Alleweldt, n.d.), so we placed a heat mat below the habitat and set it to 26°C to increase feeding activity.

Data Analysis

At the conclusion of the experimental run, we downloaded the data files from each MicroSD card and uploaded the voltage and time data for each group. We removed rows of data with excess headers or blanks. We then ran a descriptive statistical analysis of each cell in each condition. These data were used to determine the average, maximal, and minimal voltage, and the standard error for each

condition. We then performed the Shapiro-Wilk test on all three conditions to assess for normality, and finally, tested the mean voltage output of each condition to determine if our results were statistically significant. To do this, we performed a Kruskal-Wallis test on data that was non-normally distributed, and a one-way ANOVA of data that was normally distributed.

Results

We collected voltage data for the MFCs from November 14 to November 24, 2023, apart from the negative control condition. We encountered technical difficulties that resulted in premature termination of data collection for the negative control approximately halfway through the experimental run. The mean voltage produced by each MFC is summarized below in Table 1.

Cell No.	Positive Control (mV)	Experimental (mV)	Negative Control (mV)
1	13.2 ± 0.3	8.1 ± 0.3	-377.5 ± 1.4
2	8.8 ± 0.2	4.4 ± 0.3	5.9 ± 0.3
3	6.9 ± 0.2	9.0 ± 0.2	4.4 ± 0.4
4	6.0 ± 0.3	9.1 ± 0.2	8.7 ± 0.4
5	12.5 ± 0.3	5.2 ± 0.2	12.5 ± 0.5

Table 1: Calculated mean voltage with standard error for each MFC

The averaged mean voltage values for each condition were as follows: 9.5 ± 1.4 mV for the positive control, 7.2 ± 1.0 mV for experimental, and -69.2 ± 77.1 mV for the negative control. Maximal and minimal values reached by any MFC in any condition were 1,573.8 mV and -2458.5 mV (1.5738 and -2.4585 V) respectively, which were both reached by cell 1 in the negative control condition. This indicated very high variance within this MFC. We also observed that the mean voltage output for cell 1 in the negative control condition was significantly lower than those of the other MFCs in this condition, indicating high variance of cell 1 from the rest of the condition. We thus removed cell 1 from the data as an outlier and re-evaluated the averaged mean voltage for the negative control condition to obtain a

value of 7.9 ± 3.6 mV. This value, along with the averaged mean voltage for the positive control and experimental conditions, are summarized below in Figure 1.

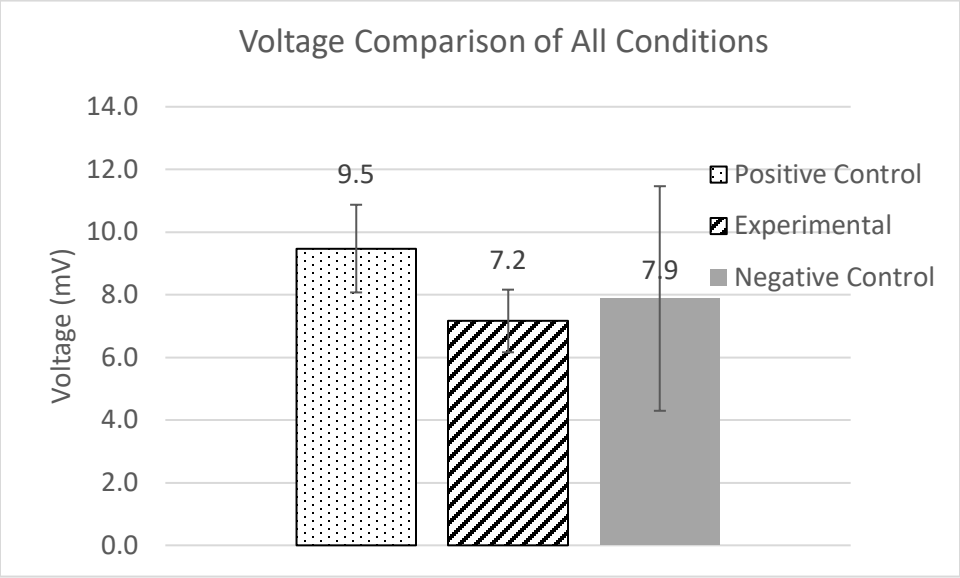

Figure 1: Comparison of averaged mean voltage of MFCs in the positive control, experimental, and negative control conditions, excluding the outlying cell (1) in negative control condition

We found that the averaged mean voltage of MFCs for the positive control, experimental, and modified negative control conditions were normally distributed, but that the intact negative control condition was not (Table 2). Because of this, the shortage of data and high variability in the negative control condition, and the small sample size for the modified negative control condition, we decided to run two statistical analyses: one between the positive control, experimental, and negative conditions, and one between the positive control, experimental, and modified negative control conditions. We then compared the acquired p-values to the critical value (0.05) to determine the statistical significance of the results, as shown below in Table 2.

Test	Condition(s)	P-Value	Significance (α = 0.05)
Shapiro-Wilk	Positive control	0.3711	Normally distributed
Shapiro-Wilk	Experimental	0.1588	Normally distributed
Shapiro-Wilk	Negative control	0.0002	Not normally distributed

Shapiro-Wilk	Modified negative control (excluding cell 1)	0.7568	Normally distributed
Kruskal-Wallis	Positive, experimental, and negative	0.3324	Medians between conditions are equal
One-way ANOVA	Positive, experimental, and modified negative	0.3927	Means between conditions are equal

Table 2: Results of statistical analyses

Discussion

Our results are not robust enough to show statistical significance between the average voltage produced by the positive control, experimental, or either negative control conditions. This means we are unable to reject our null hypothesis or draw any explicit conclusions from our analyses. The trend we observed among the three conditions is not quite aligned with our predictions, either: the positive control produced the highest average voltage, the negative control condition was in the middle, and the experimental condition produced the lowest average voltage. However, there was incredibly high variability within this group, which on average produced any where from approximately 4 to 11 mV, which is higher and lower than the maximal and minimal error ranges of the positive control or experimental conditions.

The issues we encountered with the negative control condition particularly stunt our results and make it difficult to draw meaningful conclusions. We had approximately half as much data for the negative control as for the other two conditions and found that cell 1 was a significant outlier compared to the rest of the MFCs in the group. Removing this outlier further weakened our already minimal sample size and impacted our ability to derive statistically significant results from comparisons with the other conditions. We also found that MFCs within the negative control condition, particularly cell 1, had larger variance than those in the other two conditions, which also contributed to weak statistical results. We theorize that the lack of consistent feed in the unfed negative control condition led to erratic microbial growth and electron production, as organic matter that was consumed in the substrate was

not replenished. As the consumable content of the substrate around the anode was depleted, microbes participating in the anodic biofilm were less able to produce a steady flow of electrons, instead generating voltage peaks where nutrients were available, and valleys where nutrients were not available. This pattern is likely responsible for the patchy, variable voltage we observe in the negative control condition data. We predict that regardless of MFC design the issue of variable electron production will be present for an unfed condition, resulting not in a MFC condition that does not produce voltage, but a condition that produces erratic, highly variable, and often negative voltage. This could theoretically be addressed with a longer acclimation period, which would give microbes longer to consume available nutrients in the microbial substrate to allow for the generation of a consistently low, moderately varied voltage during the experimental run.

We expect that with a larger sample size and a longer experimental run that our results could be fortified to produce more statistically significant evidence. However, further studies would also benefit from optimization of our MFC design to increase voltage output across the board. With higher voltage, we expect that the difference in average output of the MFCs would also increase and allow for more resolution between the conditions, leading to more statistically significant results. Optimization of our system should address barriers discussed in the introduction of this report, including aeration at the cathode, electrode material and surface area, and microbial community structure. We would recommend at a minimum to leave the cathodic chamber open to the air, and to use the bare stripped wire as a cathode rather than a full electrode, as was recommended by drdan152 (2013). This would increase oxygen availability and decrease internal resistance at the cathode, which would hopefully work in conjunction with other optimization strategies to increase voltage. We would also recommend that the anode have a larger surface area and potentially an alternate shape to the rectangle we created for this project to facilitate more contact between the microbial substrate and the anode. This strategy would allow for a larger biofilm to develop, hopefully translating to higher electron transfer to the anode

and increased voltage for the MFCs. Increased voltage and decreased resistance within the MFCs would also allow for current monitoring within the MFCs, which was not possible in this study due to low current production. Including this measurement would allow for MFC power output to be generated, which would facilitate an estimation of applications for this system.

To further improve upon our study design, we would suggest that future studies measure microbial substrate by mass rather than volume to ensure consistency between MFCs, and that, if possible, water not be added to the MFCs during the experimental run. Although the MFCs likely benefitted from hydration, we observed mold growth at the conclusion of the experimental run in several MFCs in the experimental condition. The addition of non-exoenergic microbes introduces competition to the microbes in the effected MFCs and may with time confound results. We therefore recommend that any water added be in small amounts (<10 mL) and mixed into the microbial substrate very thoroughly to prevent surface pooling that could result in mold growth. We also recommend that data collection methods be altered. We collected voltage data and a time stamp every two seconds in this project, which resulted in a very large dataset that was challenging to work with. We suggest the time interval between readings be increased, and that the date be recorded along with the time to allow ease of interpretation. Lastly, we would emphasize the importance of elevating and protecting electronics from potential leaks and spills in the study area. Corrosion of the negative control Arduino MEGA board resulted in severe data loss during our experimental run, which could have been avoided had the electronics been better separated from the MFCs.

Finally, we want to note that while this system is in theory an alternative to fossil fuel combustion, it still produces carbon dioxide as a product of cellular respiration and thereby plastic biodegradation. Thus, our project does not serve as a renewable alternative to fossil fuels, but a circular mechanism to biodegrade plastics on a small scale. Applications of this system could include a home garbage disposal system that could degrade plastics along with food waste and create its own power. The

MFC component would generate energy to keep the mealworms warm and aerate the cathodic chamber, meaning after an initial input of microbial substrate, water, and mealworms, the system would just have to be fed household wastes to function. It would not require any external sources of power, water, soil, or mealworms, and would be capable of degrading organic and synthetic materials alike.

Conclusion

In this project, we sought to synthesize address two issues within the scope of fossil fuel reduction: alternative sources of energy and plastic bio-depolymerization. Our goal was to create a MFC that generated energy from the frass of plastic-fed mealworms, which we tested against a 'normal', functioning MFC condition that was fed sucrose, and a non-functioning condition that was unfed. Despite the limitations in our results, and the lack of statistical significance between the conditions' mean voltage output, we were able to generate voltage in the PFMF MFC condition. This indicates that our system does generate energy, albeit in very small quantities, and that the concept of the PFMF MFC is viable for further exploration.

Supplementary Information

The Arduino code, raw data, and statistical analysis for this project can be found in a Git Hub repository at: https://github.com/casper-jm/SCI_400_Project_CJM_2023

The coordinates of the microbial substrate collection site (at Capilano University's North Vancouver campus) are: 49.3206602, -123.0184909

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since time immemorial this land would not be the incredible resource we as colonial settlers constantly take for granted. We wish to extend our most sincere gratitude and respect to the land and its Peoples.

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