ELECTROACTIVE BIOFILMS AND DEVELOPMENT OF INCUBATION CHAMBERS

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Abstract

Microbial dispersal is the process in which microbial communities move across space, away from their parent populations. This process influences the way microbial communities are structured and interact with other communities. Stainless steel meshes with varying pore size were used to physically control the dispersal of bacteria. A method to physically control and measure dispersal of bacteria was developed using Escherichia coli using meshes. These meshes were validated through inoculation of a bacterial culture inside the meshes and plating of solution that passes through the meshes. The meshes were then applied to microbial communities living in different soil types using 3D-designed, novel incubation chamber devices. In order to characterize and determine the change occurring within the communities, the 16S rRNA gene was amplified and sequenced in the microbial communities. Phylogenetic analysis was then conducted to provide the identity and proportion of bacterial species living in the respective microbial communities. This data allowed for the elucidation of microbial community change through the application of the microbial dispersal control method. Preliminary results suggest that the incubation chambers created an anaerobic environment during incubation. In future studies, these chambers must be designed allowing for an aerobic environment while maintaining sterility. The incubation chamber control method could prove practical for applications in measuring the influence of dispersal between different soil types on microbial community structure and function in future work.

Introduction

Background

Bacteria play a vital role in many of the diverse ecosystems of our planet. From fixing nitrogen to making cheese, these microorganisms have a great diversity that allows bacteria to thrive in many different environments. When studying organisms conventionally, an individual species or organism is typically studied in isolated environments. For bacteria, this proves almost impossible because bacteria are often found as part of communities. The different microbial communities often are made of extremely diverse amounts of species and have varying functions. In order to study whole microbial communities, we are required to study the holistic role of the community rather than each of the individuals independently.

Bacterial biofilms are formed virtually anywhere by communities of bacteria. These biofilms are complex surface-associated communities that provide protection for individual cells and resistance to changes and stressors in the environment. These biofilms can also have some negative characteristics such as biofouling, where microorganisms accumulate on structures and can cause severe damage or loss of function in industry. Conversely, these biofilms are also a great chance to apply their vast toughness and robustness to create bioreactors. These bioreactors can have a variety of applications such as biofuel production and waste management.

To better understand the communities of microorganisms that are responsible for these reactions and catalytic activities, the multiomic approach can demonstrate the various processes and species characteristics. This can be done by taking advantage of the central dogma of molecular biology, where the expression of proteins and functions of cells can be linked back to the information stored as nucleotide base pairs in DNA. The reading of the genome can demonstrate the various genes that are present and can have important roles in the electro activity

of the biofilm. This can then be further traced along by determining the magnitude of regulation of transcription of certain sequences and their expression in the phenotype as proteins.

Metagenomics is the study of the DNA of an entire community. We are required to use this measure in order to study bacteria since less than 2% of bacteria can be cultured in a laboratory[1]. By using metagenomics, we can determine the relative abundance of each individual species in a community. Another function of metagenomics is the identification of functional genes common within a community or between other communities. These genes can vary from antibiotic resistance genes to insulin production genes. Within a community, there are many factors driving the interactions of species and their community assemblage, one being their functional genes[2].

For these genes to propagate, species to interact and communities to form, there must be microbial interaction. One ecological process that makes a key role in how the generation and development of these microbial communities occurs and change is microbial dispersal[3]. Dispersal is a very understudied process that dynamically alters the community compositions within and between communities. There are two main types of microbial dispersal, active and passive[3]. Active dispersal is where the individual bacteria are involved in motility. Passive dispersal is where the environment is moving bacteria through space. Both of these processes have been demonstrated to affect and change biodiversity in communities. Microbial dispersal is difficult to quantify. Therefore, in this project, the community changes in diversity and composition were measured and used to determine change and dispersal.

Significance of Project

With a novel method to control and measure community dispersal, the interactions between microbial communities are able to be studied. This is especially important currently due

to the thawing of permafrost soils. As the isolated microbial communities in permafrost begin their thawing process, they become increasingly active[4]. As their activity increases, the interaction with neighbouring microbial communities increases accordingly. The effect of this interaction is difficult to study, therefore by using the method developed in this study, the study of this interaction will be facilitated and made possible.

Experimental

Electroactive Biofilms

To begin the experiment, the National Research Council will provide TRU with the biofilm samples that have been grown over a two-week period and collected at four points in triplicate from pyrite coupons. Delivered to TRU, the samples will be prepared for the various different analyses to be run.

For the metagenomic analysis, the DNA will be extracted using a DNA extraction kit. The DNA must then be isolated, and the libraries must be prepared for sequencing. A whole genome sequencing will be done in order to characterize the different species and tie their genetic information to the findings of the other analyses. The 16S rRNA gene will be amplified and sequenced as well to determine community composition.

For the metatranscriptomic analysis, a similar process will be used to the DNA extraction and isolation. However, RNA will be further purified in order to have a large quantity of mRNA relative to rRNA for sequencing.

Mesh Dispersal Test

The goal of the mesh dispersal test was to determine if the dispersal of bacteria could be limited by using meshes.

The strain of E. coli used was DH5α and contains ampicillin-resistant genes as well as green fluorescent proteins. Nutrient broth and nutrient agar plates were prepared at the producer's specifications. Both media were made with an added working concentration of 100ng/mL of ampicillin, allowing more specific growth of our bacterium. The bacterium was inoculated from frozen beads into a culture of nutrient broth and a nutrient agar plate. Another culture was inoculated with a single isolated colony of this bacterium and allowed to incubate at

37.0°C for 16 hours. This culture was then used as our starter culture in log phase for the experiment.

Using the prepared log phase culture, a small volume of 1.000mL was inoculated into 100mL of nutrient broth in an Erlenmeyer flask. Using a calibration curve (Figure 1) made in a previous experiment, the approximate goal concentration was determined to be above 0.5 OD_{600} absorbance units. This culture was allowed to incubate at 37.0°C and the OD_{600} was monitored periodically using a spectrophotometer until the bacteria were above 0.5 absorbance units.

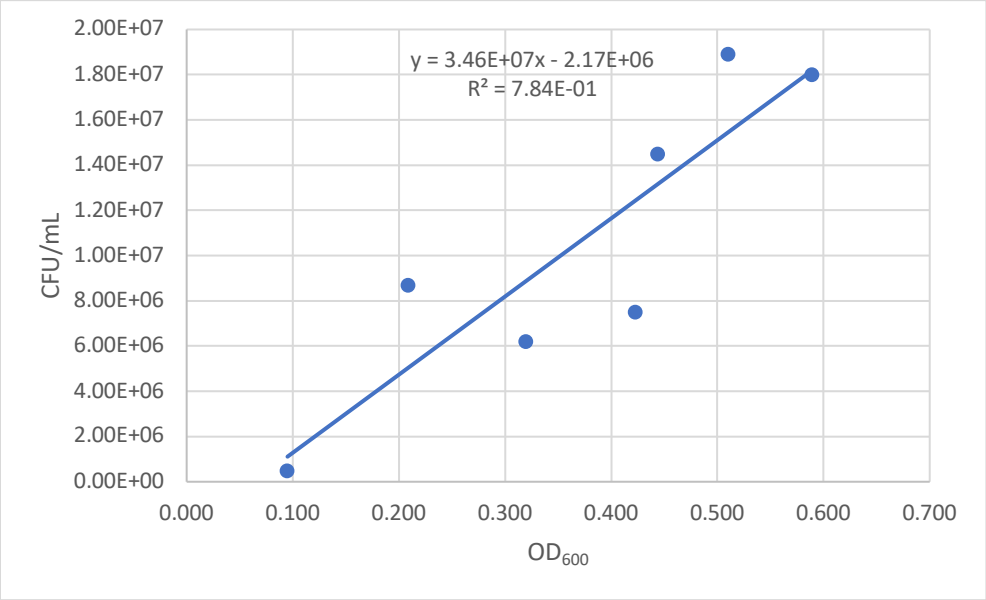

Figure 1. Calibration curve of CFU/mL plotted against OD_{600} of Escherichia coli.

Three different sized meshes were used in this experiment, 1µm, 25µm, 75µm. These were made into an open-top, 6.8” height x 8.6” circumference, cylindrical shape and sealed with epoxy adhesive as well as cold-weld steel reinforced epoxy. These meshes were placed in a hop filter for structural support and the whole device was placed in a 1L beaker.

For the experiment, the autoclaved beakers were filled with 500mL of sterile nutrient broth and stored at 4°C. A sterile stir bar was placed inside the beaker in order to allow for

movement of the solution throughout. The middle of the mesh was inoculated with the >0.5 OD_{600} bacteria to begin.

The bacteria were allowed to diffuse throughout the beaker and the solution outside the mesh was sampled at various time points. For the 1µm mesh, the solution was sampled at 1, 5, 10, 20, 30 minutes. For the 25 and 75µm meshes, the solution was sampled at 1, 2.5, 5, 7.5, 10 minutes. The sampled solutions were serially diluted and plated in triplicate in order to determine the colony forming units per mL that served as a measure of concentration. The original inoculant solution was also plated to give us a baseline and to determine what the maximum concentration of bacteria that could be present in the beaker.

Soil Interaction with Limited Dispersal

The goal of the soil interaction test was to determine if a device and method to control community dispersal could be constructed and utilized.

Two soils were collected from different ecosystems in Kamloops, BC. The first was a grasslands ecosystem where the soil was dryer and in a warm environment. The second was a forest ecosystem where the soil contained more water and a cooler climate. Both of these soils were collected using aseptic technique into a plastic bag. Following collection, the soils were sifter using a soil sift and stored in a 4°C fridge until used.

The soil composition was analyzed prior to any manipulation. This included water content, bulk weight and particle density. These metrics were used to determine the amount of water necessary to reach 95% saturation of the soils. Soil samples were either treated with regular deionized and filter-sterile water or nutrient water was used from the opposing soil type. The nutrient waters were extracted from each of the soils through a vortex technique. The water

was collected following a spin-down and collection of supernatant containing the dissolved nutrients. This solution was filter-sterilized before use.

DNA extractions were conducted in triplicate of each soil type prior to any manipulations in order to conduct metagenomic analysis of the microbial communities.

The soils were then soaked to 95% saturation with either normal or nutrient water. When homogenously wet, soils were then placed into incubation chambers (Figure 2). These chambers were designed and 3D printer using the TRU Makerspace. The chambers consisted of two cylindrical screw-on tops with a mesh filter serving as a barrier between them. These mesh filters were the same as used in the Mesh Dispersal Test. The placement of the soils followed the variables listed in Table 1.

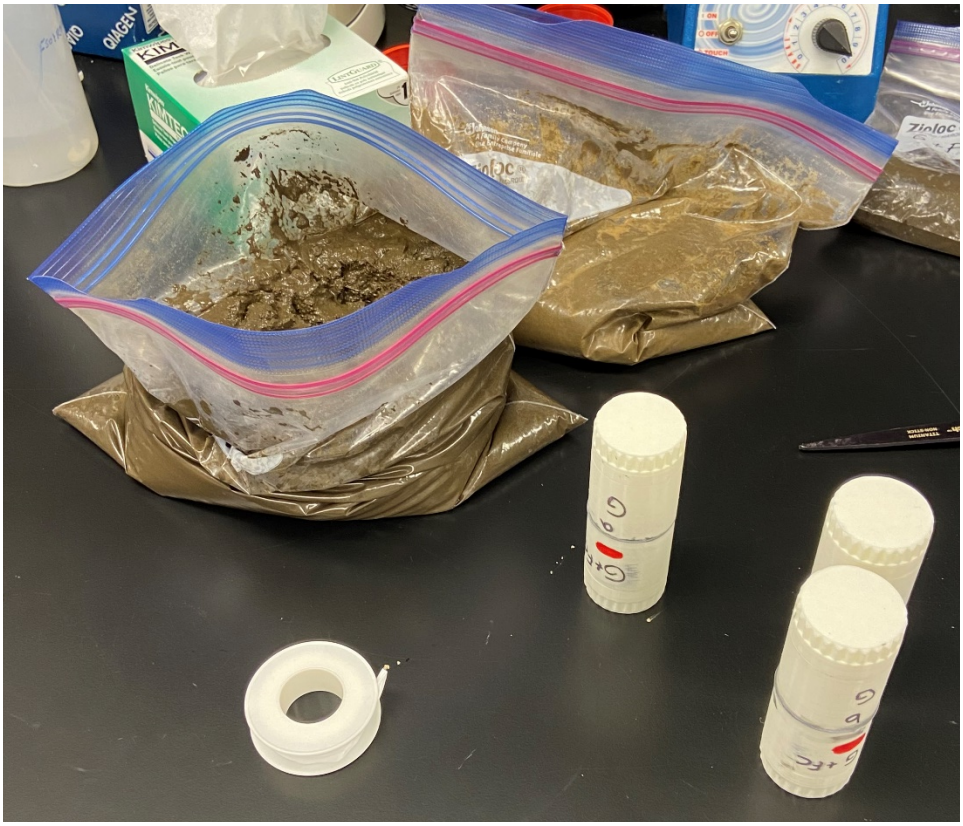

Figure 2. Incubation chambers.

Table 1. Incubation chamber soil characteristics.

Filter Pore Size	Soil Type on Top	Soil Type on Bottom	Triplicate Number
1µm	Forest soil	Forest soil	A
25µm	Forest soil with grasslands nutrients	Forest soil with grasslands nutrients	B
75µm	Grasslands soil	Grasslands soil	C
	Grasslands soil with forest nutrients	Grasslands soil with forest nutrients	

The chambers were sealed to be airtight and sterile preventing contamination. The chambers were also flipped evenly on either side to prevent biased movement of nutrients favoured by gravity. Following 20 days of incubation, the DNA was extracted from all chambers to determine the change following the treatment.

For the DNA extractions, the DNeasy Power Soil Pro kits were used to obtain ample DNA from the samples. Following the extractions, PCR was run with primers amplifying the 16S rRNA gene. The amplification was verified by using gel electrophoresis shown in Figure 3 below. Following amplification, the DNA solution was cleaned using magnet beans to purify the DNA and remove byproducts and contaminants. These solutions were then sequenced using next-generation sequencing by Breanne McAmmond and the output processed by Dr. Jonathan Van Hamme. The data was then analyzed in R Studio.

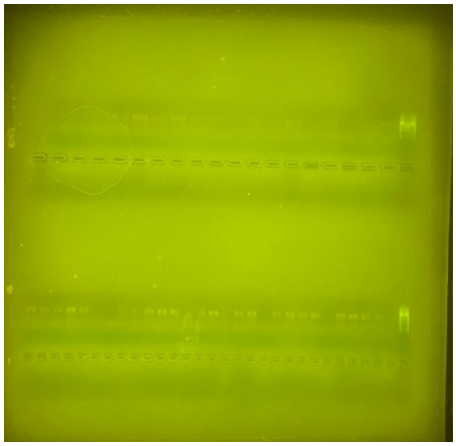

Figure 3. Image of cleaned PCR products.

Results and Discussion

A literature review of electroactive biofilms was conducted. The methods of analysis were determined and studied as well as their potential impacts. Issues arose when collecting electroactive biofilm samples. These lead to our contributor to be unable to provide the samples we needed to conduct analysis and experimentation. These samples are still under development and baseline DNA extractions have been done with successful sequencing of 16S rRNA data for future analysis.

The Mesh Dispersal Test demonstrated the ability of the stainless steel meshes to control the dispersal of bacteria successfully. This was demonstrated due to higher concentrations of bacteria in larger pore sized meshes compared to smaller pore sizes (Figures 4-6).

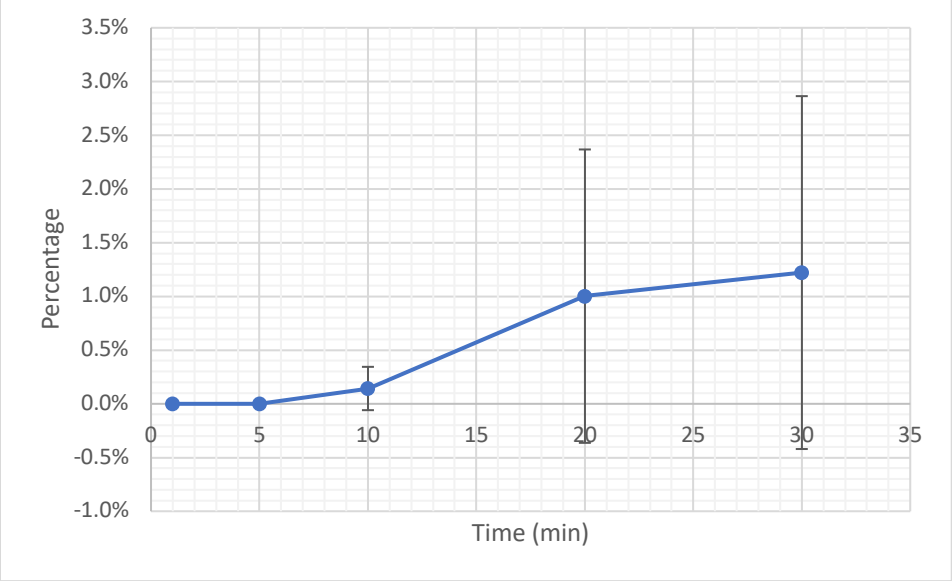

Figure 4. Percentage of inoculated bacteria trespassing the 1µm mesh plotted over time (n=3).

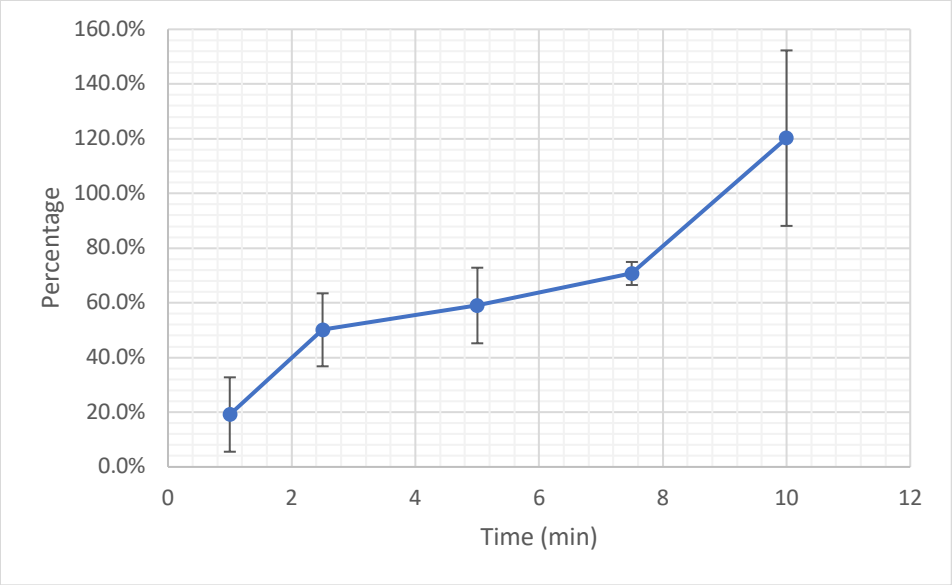

Figure 5. Percentage of inoculated bacteria trespassing the 25µm mesh plotted over time (n=3).

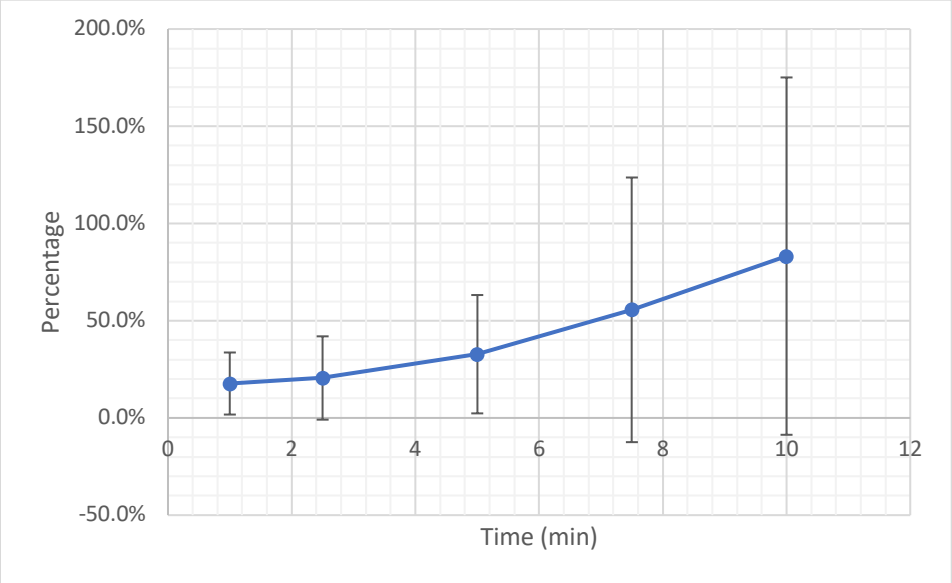

Figure 6. Percentage of inoculated bacteria trespassing the 75µm mesh plotted over time (n=3).

These results demonstrate the capacity of these stainless steel meshes to limit microbial community dispersal. However, further extending this to incubation chambers proved difficult. This difficulty arose from poor design as there was artificial selection of anaerobic bacteria in the chambers. Through making the chambers fully contamination-resistant and airtight, no air

exchange was allowed. By not allowing any oxygen entry into the chambers, there was no longer the capacity for aerobic bacteria to survive. However, anaerobic bacteria, who do not need oxygen and thrive in low to non-oxygenated environments, thrived in these chambers and reproduced heavily. This huge push for anaerobic bacteria greatly overpowered the aerobes which completely shifted the community composition. Therefore, we were unable to determine the interaction between two soils in the chambers since the amount of anoxic effect were what varied between samples.

These assumptions can be concluded by the analysis of the MDS plot below (Figure 7), which demonstrates sample difference by distance between points.

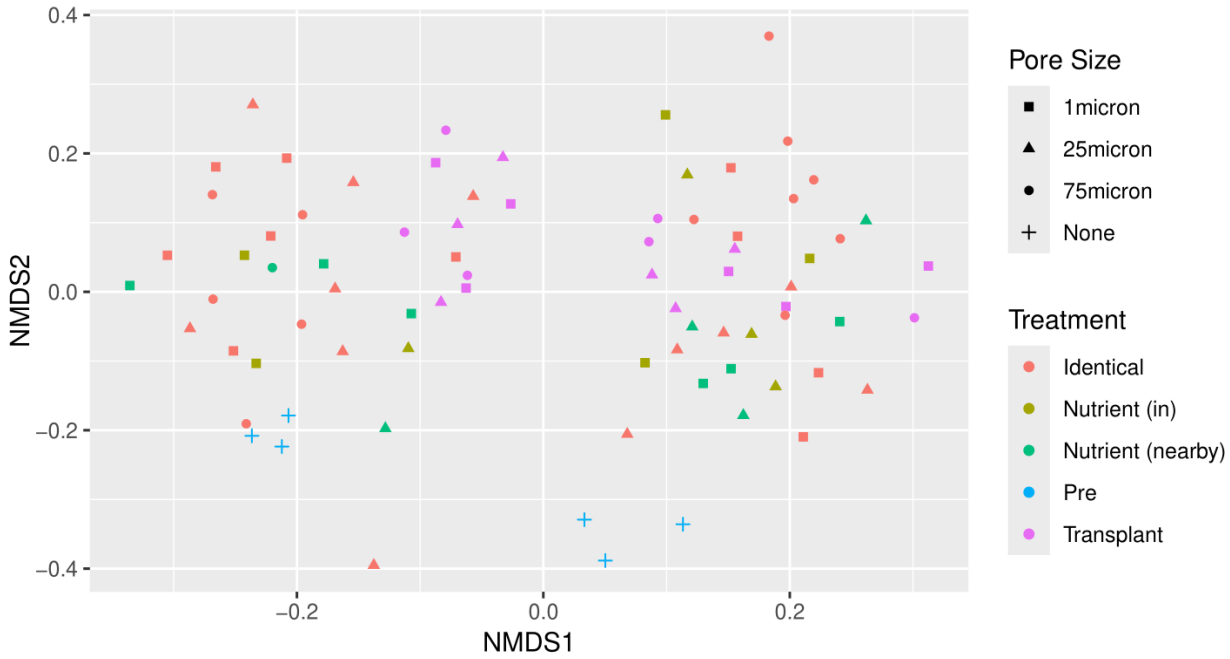

Figure 7. MDS plot demonstrating the Bray-Curtis dissimilarity of samples before and after each treatment.

The plot shows the Pre samples with no treatment in blue. The complete positive shift in both directions symbolizes a general shift to everything in the chambers. This can be shown due to the controls in the chambers that also shifted. To further confirm this, the samples in the

positive directions were analyzed further and it was seen that their anaerobic composition was much greater than baseline.

The project data allowed for the demonstration of microbial community change through the application of the microbial dispersal control method. While the anaerobic effect was strong in the chambers, the meshes were validated to showing dispersal control.

Future Work

The collection of proper samples that can be utilized for DNA extractions is necessary for the in depth analysis of electroactive biofilms. Therefore, having a secure system in place to deliver these samples is needed.

For future studies, there are multiple considerations and improvements that can be made to this research. Firstly, the incubation chambers can be redesigned. By doing so, the chambers should be produced in a manner that allows air flow and oxygen entry. This subtle change is difficult due to the limitation and risk of contamination that would significantly affect the results. If done successfully, the aerobic environment would not be artificially selecting for anaerobic bacteria.

Finally, the method should undergo precision studies such as interday, intraday and continuous studies to ensure its precision and relationship to dispersal. By conducting these studies, the method can be validated and demonstrated to be reliable and capable.

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