Optimization of Biophotovoltaic Devices
An Analysis of the Effects of Cyanobacteria and Electron Transport Methods

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Research Goal

The goal of this project is to analyze the efficiency of different models of BPVs, including different bacteria strains and electron transfer mechanisms.

Rationale

Finding alternative forms of energy has been a goal of many scientists over the years. From wind energy to solar panels, there are many different alternative forms of energy. However, these forms of energy often require a lot of upkeep. Biophotovoltaic (BPV) utilize the photosynthetic properties of microalgae and other aerobic photosynthetic bacteria to harvest energy. An added benefit of this device is that the microorganisms are self-sustaining and can produce energy even in the dark without much interference. However, the output of a BPV has not yet met the standards of current photovoltaic devices, decreasing its viability as a future energy source. This project aims to examine the efficiency of various models of biophotovoltaics in order to see which model would produce the highest voltage output. Different strains of microalgae and different modes of electron transport to the anode were tested. For direct transport, the algae was grown as a biofilm on a conductive surface, while indirect transport systems utilized potassium ferrocyanide as an electron transfer mechanism between the anode and the algae. It was concluded that the direct electron transfer devices provided a higher voltage output, with slight differences between each type of microalgae. In the future, additional optimization of this device could lead to implementation within green roofs along with wastewater and desalination applications.

Introduction

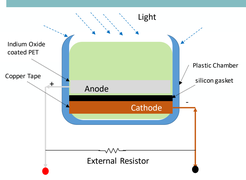
In recent years, there has been an increased importance placed on alternative forms of energy. As the threat of climate change looms, scientists are looking towards finding a way to create energy without harming the environment. Solar cells, or photovoltaics, is an important method of renewable energy that has emerged. However, this form has its drawbacks, and scientists continue to look for future methods of renewable energy. A promising solution has been found in the idea of bioelectrochemical systems (BES). BES systems, contrary to a photovoltaic system, has the ability to repair itself and can produce power in the dark. The distinguishing feature of a BES is that they use photosynthetic organisms to convert light energy into electricity. This study focuses on a specific type of BES, known as a biophotovoltaic cell (BPV). Biophotovoltaic cells directly use an oxygenic photosynthetic organism to generate electricity. The most common organisms used include algae and cyanobacteria, due to their photosynthetic properties.

There are two main ways that electron transfer occurs in the BPV. The transfer can either be indirect, or direct. In an indirect transfer, electrons are transported by carriers. However, carriers can negatively impact the amount of energy produced as there are limitations is mass transfer to electrons. There are many different chemicals that can be used as a carrier, and this project utilized potassium ferricyanide. While some bacteria excrete electron mediators, potassium ferricyanide must be added to the BPV and as such is known as an artificial electron mediator. This chemical is able to accept electrons from transmembrane proteins within the cell itself. However, these chemicals can break down in solvents, and can pose dangers of toxicity to the environment.

In order to produce a direct transfer of electrons, the organisms must be in direct contact with the anode, and transfer occurs more efficiently. However, the only way to achieve direct transfer is to grow a biofilm directly onto an anode. The intracellular mechanisms that make this transfer of electrons possible are complex and can vary from each device. Specific strains of bacteria, such as the Cyanobacteria *Synechocystis* tested in this project, produce nanowires. These nanowires are formed from pili or the outer membrane of a cell and allows for cells in an anoxic environment to use oxygen as an electron acceptor. An electron acceptor is an oxidizing agent that is reduced as it accepts electrons. Therefore, through the formation of nanowires, bacteria like *Synechocystis* is able to produce more electrons in a thick biofilm environment. While other bacteria do not produce these nanowires, they display special properties while in a biofilm environment. Electrons travel to the anode through redox proteins at the surface of the cell. When chemicals are oxidized and reduced at these sites, electrons can travel between the cell and the anode surface. In addition, biofilm formation, which is integral for direct electron transport, is complex and has multiple steps.

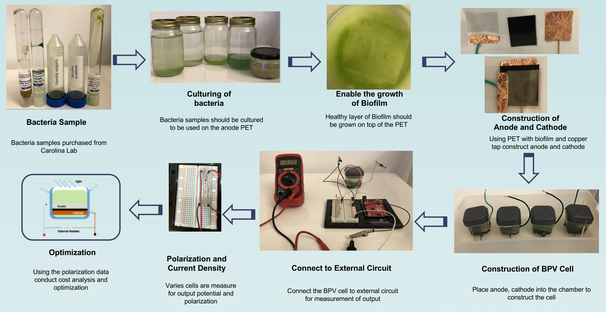
The first step of biofilm formation is when cells begin to adhere to a surface. In order to stay adhered, they excrete a substance known as extracellular polymeric substance. This substance is a mix of sugar, phosphates and nucleic acids. After the primary cells have adhered, more bacteria grow on top, creating a complex structure with paths for the transfer of nutrients and electrons. Due to their structure, they also have potential to be used as a filter as they stop the flow of other particles. The thickness of a biofilm, however, can lead to slower growth as cells may have less access to nutrients needed for growth. After the biofilm grows and matures, sections of the biofilm may detach and disperse into the surroundings. This cycle of biofilm formation and dispersion continues in suitable environments.

Biophotovoltaic (BPV) Cell Design



Fuel cell design should be focused on creating a prototype that is scalable, simple, reproducible and cost effective. Copper tape was used to connect wires to the anode to limit surface area contact of the coated PET anode. The design was kept simple with only 4 key materials – Plastic Chamber, Copper Cathode, silicon gasket and Coated PET. Cost of single fuel cell was only $5.50 prorated.

Research Execution Method



* All Images and Graphs by Aakriti Lakshmanan

Culturing of Algae

The algae was purchased from Carolina Biological, and came in 30 mL of solution. In order to sub-culture the cyanobacteria, begin by sterilizing all items that come into content with the algae. Pour 16 ounces of sterile Alga-Gro medium into the glass jars. Using a sterile pipet, add 10 mL of the solution in the test tubes purchased. Close the jars and allow the algae to grow for 7-10 days under 100 foot-candles of light on 12-hour dark and 12-hour light cycle. To continue algae growth, bacteria was streaked onto Petri dishes and used for another subculture.

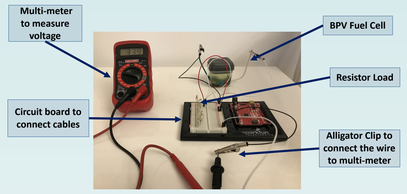
Growth of Biofilm on Anode Material

The biofilm was grown by pipetting the bacteria cells on to the PET conductive layer and leave to settle for 60 minutes. The anode was then transferred to fresh medium and allowed to mature and grow for 7 days. The Indium Oxide coated PET plate was visually inspected to ensure the growth of biofilm. Cell density can then be determined by count or change in color of clear PET layer to ensure the anode has enough biofilm formation to conduct the experiment.

Construction of BPV Cell

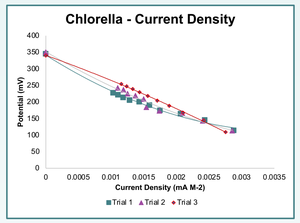
To begin, construct the cathode using copper tape and copper wire. Copper tape acts as a good conductor and area for the redox process to occur. The next layer is the silicone gasket which act as semi-permeable layer between the anode and the cathode. The anode is made with Indium Oxide coated PET. Copper tape to used to connect a copper wire to the anode, since the surface cannot be soldered. A biofilm has been already cultured on the coated side of the PET anode. The chamber with finally filled with medium to allow continuous growth of bacteria.

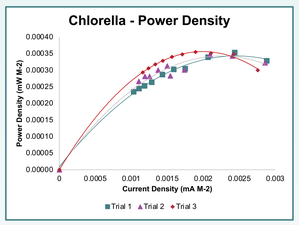
Power Density Data Collection

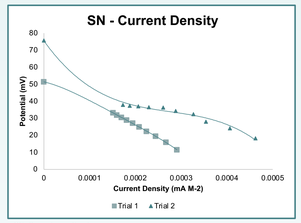


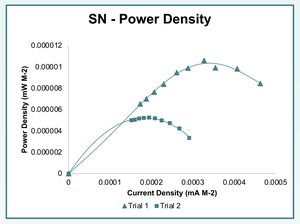
To ensure accuracy of the measurement, the distance between anode and cathode should be constant. Therefore, the cell was not moved while collecting data. Using an alligator clip, the positive and negative terminals of the cell were connected to the breadboard. The wires were connected to an external resistor that was gradually increased. The other end was connected to digital multi-meter to measure the voltage from the operational BPV Cell. Data was documented in Excel to complete data analysis.

Power Density Data and Results









SN- **Synechocystis nigrescens**

Chlorella – Trial #1 Data

Voltage/Potential	Resistance (Ohms)	Current (Amps)	Power (Watts)	Power Density (mW M-2)	Current Density (mA M-2)
345	Open Circuit Voltage (OCV)				
114	19540	5.83419E-06	0.00000066510	0.00033	0.00288
145	29400	4.93197E-06	0.00000071514	0.00035	0.00244
164	39200	4.18367E-06	0.00000068612	0.00034	0.00207
174	49100	3.54379E-06	0.00000061662	0.00030	0.00175
190	59000	3.22034E-06	0.00000061186	0.00030	0.00159
200	68900	2.90276E-06	0.00000058055	0.00029	0.00143
205	78800	2.60152E-06	0.00000053331	0.00026	0.00128
213	88700	2.40135E-06	0.00000051149	0.00025	0.00119
221	98500	2.24365E-06	0.00000049585	0.00024	0.00111
227	108400	2.0941E-06	0.00000047536	0.00023	0.00103

Chlorella – Trial #2 Data

Voltage/Potential	Resistance (Ohms)	Current (Amps)	Power (Watts)	Power Density (mW M-2)	Current Density (mA M-2)
350	Open Circuit Voltage (OCV)				
113	19540	5.78301E-06	0.00000065348	0.00032	0.00286
143	29400	4.86395E-06	0.00000069554	0.00034	0.00240
166	39200	4.23469E-06	0.00000070296	0.00035	0.00209
173	49100	3.52342E-06	0.00000060955	0.00030	0.00174
184	59000	3.11864E-06	0.00000057383	0.00028	0.00154
209	68900	3.03338E-06	0.00000063398	0.00031	0.00150
219	78800	2.77919E-06	0.00000060864	0.00030	0.00137
225	88700	2.53664E-06	0.00000057074	0.00028	0.00125
237	98500	2.40609E-06	0.00000057024	0.00028	0.00119
242	108400	2.23247E-06	0.00000054026	0.00027	0.00110

Chlorella – Trial #3 Data

Voltage/Potential	Resistance (Ohms)	Current (A)	Power (Watts)	Power Density (mW M-2)	Current Density (mA M-2)
340	Open Circuit Voltage (OCV)				
109	19540	5.57830E-06	0.00000060803	0.00030	0.00275
144	29400	4.89796E-06	0.00000070531	0.00035	0.00242
167	39200	4.2602E-06	0.00000071145	0.00035	0.00210
188	49100	3.82892E-06	0.00000071984	0.00036	0.00189
204	59000	3.45763E-06	0.00000070536	0.00035	0.00171
218	68900	3.16401E-06	0.00000068975	0.00034	0.00156
229	78800	2.90609E-06	0.00000066549	0.00033	0.00144
239	88700	2.69448E-06	0.00000064398	0.00032	0.00133
247	98500	2.50761E-06	0.00000061938	0.00031	0.00124
254	108400	2.34317E-06	0.00000059517	0.00029	0.00116

SN– Trial #1 Data

Voltage/Potential	Resistance (Ohms)	Current (A)	Power (Watts)	Power Density (mW M-2)	Current Density (mA M-2)
51.4	Open Circuit Voltage (OCV)				
11.5	19540	5.88536E-07	0.00000000677	0.00000	0.00029
15.9	29400	5.40816E-07	0.00000000860	0.00000	0.00027
19.4	39200	4.94898E-07	0.00000000960	0.00000	0.00024
22.3	49100	4.54175E-07	0.00000001013	0.00001	0.00022
24.9	59000	4.22034E-07	0.00000001051	0.00001	0.00021
27.1	68900	3.93324E-07	0.00000001066	0.00001	0.00019
28.9	78800	3.66751E-07	0.00000001060	0.00001	0.00018
30.5	88700	3.43856E-07	0.00000001049	0.00001	0.00017
31.8	98500	3.22843E-07	0.00000001027	0.00001	0.00016
33.2	108400	3.06273E-07	0.00000001017	0.00001	0.00015

SN– Trial #2 Data

Voltage/Potential	Resistance (Ohms)	Current (A)	Power (Watts)	Power Density (mW M-2)	Current Density (mA M-2)
75.7	Open Circuit Voltage (OCV)				
18.3	19540	9.36540E-07	0.00000001714	0.00001	0.00046
24.2	29400	8.23129E-07	0.00000001992	0.00001	0.00041
28.1	39200	7.16837E-07	0.00000002014	0.00001	0.00035
32.5	49100	6.61914E-07	0.00000002151	0.00001	0.00033
34.4	59000	5.83051E-07	0.00000002006	0.00001	0.00029
36.4	68900	5.28302E-07	0.00000001923	0.00001	0.00026
36.6	78800	4.64467E-07	0.00000001700	0.00001	0.00023
37.1	88700	4.18264E-07	0.00000001552	0.00001	0.00021
37.4	98500	3.79695E-07	0.00000001420	0.00001	0.00019
37.9	108400	3.49631E-07	0.00000001325	0.00001	0.00017

Statistical Analysis

Anova: Two-Factor Without Replication - Chorella

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows (Voltage for Different Resistances)	3093.891	10	309.3891	22.24802	**0.002%**	2.978237
Columns (Voltage Recorded from Each Trial)	470.1314	1	470.1314	33.80692	**0.017%**	4.9646027

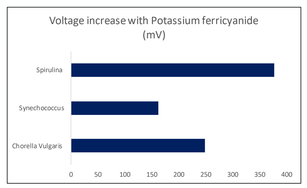
Anova: Two-Factor Without Replication - Synechocystis Nigrescens

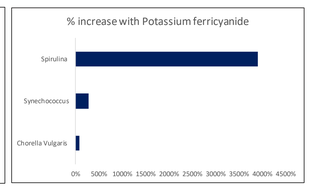
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows (Voltage for Different Resistances)	111506.7	10	11150.67	219.449	**0.00%**	2.347878
Columns (Voltage Recorded from Each Trial)	907.0909	2	453.5455	8.92593	**0.17%**	3.492828

Indirect Transfer Data

Bacteria Type	Resitance	Without PF (mV)	With PF (mV)	Delta	% Delta
Chlorella Vulgaris	OCV	345	610	265	77%
	108400	227	458	231	102%
Synechococcus	OCV	75.7	230	154	204%
	108400	37.9	207	169	446%
Spirulina	OCV	10.9	389	378	3469%
	108400	8.4	385	377	4483%

PF= Potassium ferricyanide





Biochemical Process

The process of oxidation and reduction that occurs in the BPV has various steps, and electron transfer mechanisms play a big role in this process. During photosynthesis, light is absorbed by chlorophyll pigment molecules, which energizes their electrons. These electrons are then sent to the thylakoid membrane. These chlorophyll molecules then borrow electrons from water molecules to make up for the prior loss of electrons. During this process, the water molecules is broken up. The specific equation is mentioned below:

$$2H_2O \rightarrow O_2 + 4H^+ + 4e^-$$

Potassium ferricyanide accepts these electrons and ionizes into potassium ferrocyanide.

$$4e^- + 4[Fe(CN)_6]^{3-} \rightarrow 4[Fe(CN)_6]^{4-}$$

When potassium ferrocyanide reaches the anode, it transfers the four electrons to the anode and turns back to potassium ferricyanide. When the cathode and anode are connected to an external load, electrons can travel to the cathode and complete the reaction shown below, ending with the final product of water. This process repeats and creates a voltage output that can then be used for other applications.

$$O_2 + 4H^+ + 4e^- \rightarrow 2H_2O$$

The main difference between a biophotovoltaic device and a microbial fuel cell can be found at the electrochemical level. In a microbial fuel cell, heterotrophic organisms in an anaerobic environment oxidize organic carbon. In contrast, photosynthetic organisms in a BPV utilize light to break down water in an aerobic environment.

Data Analysis

Output from BPV fuel cells were measured at varies external resistance level to draw the polarization curve. Open-circuit voltage (OCV) measured three different types of Cyanobacteria showed the Chlorella Vulgaris with highest at ~345 mv with constant output at the same level while the Spirulina with lowest at 10.9 mV well below the control fuel cell (without any bacteria) at 15.7 mV.

As the external resistance changed, the voltage produced by the BPV was affected. Power density is a measurement of the amount of power in watts that can be created in a surface area of one square meter. Using density instead of the actual value makes sit easier to predict how the cell would perform at a larger scale. Current density is a similar value, but instead focuses on the amount of current produced in a surface area of one square meter. The graphs of the power density showcase an important value. The power density cure resembles an inverted u shape. The maximum power density value occurs when the external resistance is equal to the internal resistance, and allows for the cell to be used in maximum power production conditions. This curve is commonly known as a polarization curve.

When the data is analyzed, it is clear that there are some key differences between the different kinds of bacteria. When Chlorella vulgaris was tested, trial 1 had an optimal power output of 0.0035 mW per square meter. This value occurred when the external resistance was 24900 Ohms. Therefore, the cell likely has an internal resistance close to that value. This internal resistance can be impacted by a multitude of variables, such as the distance between the anode and the cathode, which highlights the need for an experimental environment devoid of these differing factors. The relationship between the current and voltage potential was almost linear for trial 1, with an open circuit voltage ranging from 340 to 350 millivolts for all of the trials. When Synechocystis nigrescens was tested, the values of current, density, and voltage potential were lower. This main cause of this would most likely be a weaker biofilm attachment and formation. The optimal power output recorded was 0.0003268 mW per square meter. The two trials of this bacteria also produced varying results, which highlights the need for addition repetitions of this experiment.

An Anova test was conducted on the data collected. The p-values for Chlorella and Synechocystis respectively was 0.017 and 0.17. As these values are lower than 0.5, the data is statistically significant. The indirect transport of electrons also produced a different voltage output. For chlorella, the open circuit voltage was doubled to a value of 610 mV. For Synechocystis, the open circuit voltage was increased to 230. An addition cyanobacteria, spirulina, was introduced in this experimental section and achieved an open circuit voltage of 389 mV. While the voltage potential was significantly increased, the toxicity of potassium ferricyanide and the potential for ionization reduces the applications of this electron transport mechanism.

Conclusions

In total, the cell with the chlorella biofilm with an added indirect electron transfer mechanism produced the highest voltage in an open circuit. During the research, we tested the efficiency of different strains of microalgae and different modes of electron transport to the anode. With relatively simple construction and low cost materials, we were able to produce average of 400 mv from small fuel cell. For direct transport, the algae was grown as a biofilm on a conductive surface, while indirect transport systems utilized potassium Ferro cyanide as an electron transfer mechanism between the anode and the algae. Chlorella readily formed biofilms when compared to the other strains, and biofilm growth was seen visually within 24 hours of culturing, as results Chlorella Vulgaris performed well with direct transfer method with 345 mV output, while addition of Potassium ferricyanide increased the output for the Chlorella Vulgaris the output normalized back to 350 mV after few hours.

Spirulina was originally planned to be used for both indirect and direct electron transfer, but biofilm formation did not occur so the initial output was only 10.9 mV OCV. Further research reveals that spirulina actually displays anti-biofilm qualities, and as such not all cyanobacteria are viable for use in these cells. Addition of Potassium ferricyanide show an immediate improvement of 3000% increase with output at 378 mV OCV. Unlike Chlorella Vulgaris, the output remained at the level and had limited impact to health of bacteria. BPV Fuel cell works both during day and night so the BPV Cell could be a great alternate where other energy sources were not viable. In the future, additional optimization of this device could lead to implementation within green roofs along with wastewater and desalination applications. In this project, a greater understanding of the biochemical processes and the optimal condition for a biophotovoltaic cell was gained. Future goals of this project include analyzing different anode and cathode orientations, as well as quantifying biofilm growth.