

# The Development and Maximization of a Novel Photosynthetic Microbial Fuel Cell Using *Rhodospirillum rubrum*

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## Summary

This study analyzes the potential viability of the photosynthetic bacterium *Rhodospirillum rubrum* for producing electricity via microbial fuel cell (MFC), as prior research has not investigated this capacity. A prototype for a photoMFC was developed using clear PVC along with carbon cloth and steel electrodes. Initial testing revealed that *R. rubrum* could produce power utilizing the photoMFC (peak power of approximately 1.25 W/m<sup>2</sup>). Having established *R. rubrum*'s capacity for photoMFC performance, the wavelength of exposed light and resistance were modified to determine the ideal conditions. An analysis of variance (ANOVA) revealed that the differences in power outputs under varied wavelengths were statistically significant ( $p < 0.0001$ ). Power curves were calculated to determine the optimal resistance via regression analysis ( $r^2 = 0.93$ ,  $p < 0.0001$ , optimized resistance: 231  $\Omega$ ). The fuel cell was lastly monitored under sunlight (in a greenhouse) over a 10-day trial, with results showing that the photoMFC could perform effectively under practical outdoor conditions. Under optimal conditions, the *R. rubrum* photoMFC was predicted to produce maximum instantaneous power of 1.25 W/m<sup>2</sup>. In comparison to other high-power output photoMFCs, the *R. rubrum* photoMFC performed about 44% as effectively. Although the *R. rubrum* photoMFC did not perform as efficiently as other reported photoMFCs, its abundance in facilities that invite practical MFC implementation such as wastewater treatment coupled with the fact that *R. rubrum* is both a heterotrophic and photosynthetic bacteria support its usefulness in realistic, large-scale industrial MFC models.

## Introduction

In 2012, over 7 billion people lived on the planet (1). By 2050, this number is expected to increase to 9.4 billion (2). There is worry that in the future, energy demand will exceed energy supply. Present sources of energy are largely crude, inefficient, and environmentally harmful, evidenced by rising temperature and carbon dioxide levels (2). Fossil fuels, for example, constitute over 75% of the world's energy consumption, but scientists do not expect it to meet the growing global demand for energy; oil reservoirs alone are projected to run out within ten to twenty years (3). While the global energy consumption in 2012 was around 13.5 terawatts(TW)/year, by 2050 that figure is projected to increase to 41 TW/year, three times its present level (4). Without an alternative solution, humankind's energy needs will be severely limited in a deleterious fashion. Thus greener, more sustainable, and cost-effective alternative methods must be investigated to solve this impending dilemma.

First engineered by M.C. Potter in 1911 (5), the microbial fuel cell (MFC) represents a currently promising area of alternative energy, namely bio-electricity. The concept of a microbial fuel cell (Figure 1) is similar to that of a chemical fuel cell in that both employ an anode (which accepts electrons) and a cathode (which donates electrons). Chemical fuel cells are electrochemical devices that regulate the flow of electrons ( $e^-$ ) and protons ( $H^+$ ) through redox reactions (6). Microbial fuel cells require live bacteria to donate the electrons necessary for current. The bacterial oxidation of the organic substrate allows electrons to circulate through a series of respiratory enzymes in the bacterial cell (NADH Dehydrogenase, Cytochrome b-c1, Cytochrome Oxidase, ATP-Synthase), thereby going through the process of respiration and creating energy for the bacteria in the form of ATP (7). Typically, in the final stage of this process, electrons are donated to a terminal electron acceptor (TEA), which is thus reduced. Common TEAs include oxygen, nitrate, and sulfate (8). By placing the bacteria in a completely anoxygenic environment (the anode chamber) and replacing the natural TEA with an artificial one (carbon cloth or graphite) called the anode, it is possible to collect terminal electrons, thereby generating current.

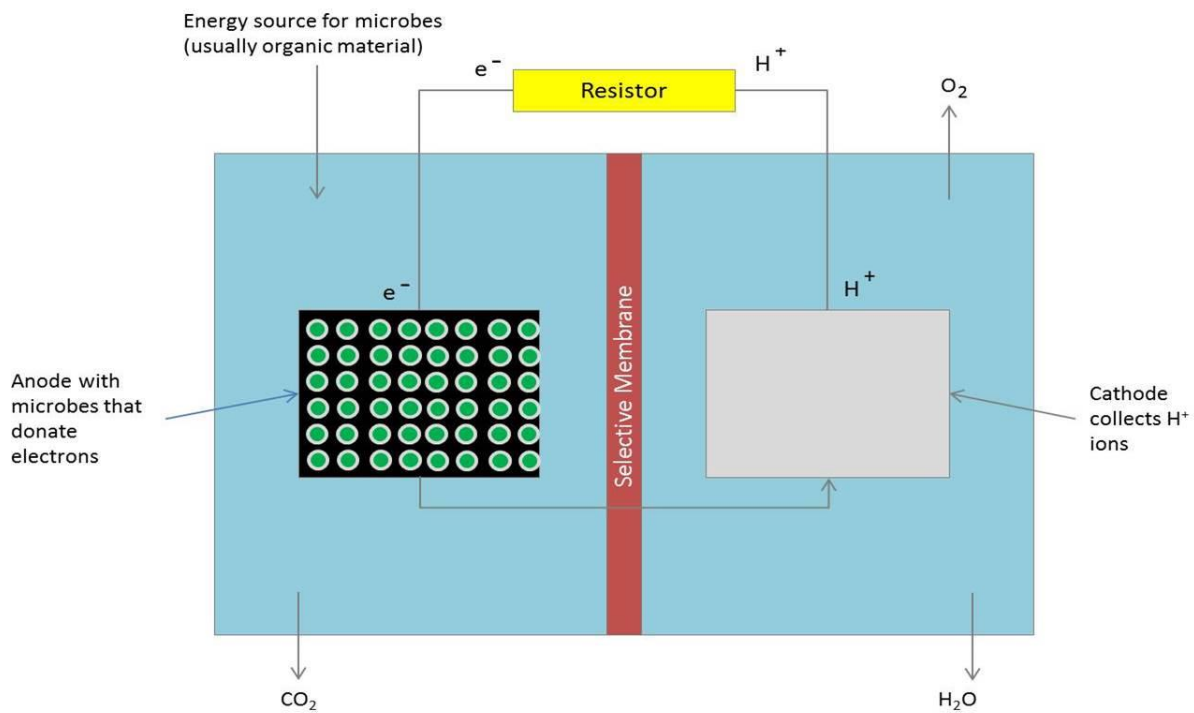


Figure 1: The mechanics of the microbial fuel cell. Bacteria grow in the anode chamber on a carbon-based anode; these bacteria donate electrons to the anode. Hydrogen ions generated during respiration travel across a selectively permeable membrane into the cathode chamber, where they are picked up by a (usually) metal cathode submerged in a conductive solution. The anode and cathode are connected externally over a load (a resistor) forming a circuit.

In addition to electrons, a complete circuit must allow protons to flow across chambers driven by a potential gradient. During the electron transport chain in the bacteria, protons are also released. These ions diffuse through a selectively permeable cation exchange membrane (CEM) into the cathode chamber, where they are collected by a conductive metal (the cathode) suspended in a NaCl solution.

Not all bacteria can transfer electrons from inside the cell membrane to the outside environment. A special category of bacteria called exoelectrogens alone are able to transfer electrons exogenously, which make this group a convenient bacterium to use in an MFC. Non-exoelectrogenous bacteria can “artificially” transfer electrons through a mediator, most often in the form of a liquid, to produce a usable current through the harnessing of electrons unbound from the electron donor (8). However, many chemicals used as mediators such as ubiquinones, dyes and metal complexes (9, 10) can be unsafe to handle, environmentally unsafe, and expensive.

Recently, the breakthrough of mediator-less MFCs attracted newfound attention (2). Even so, many details concerning the practicality and mechanics of the current MFC are still largely unknown.

Heterotrophic bacteria like *Escherichia coli*, which are usually used by MFCs in the anode chamber, renders this method only practical in situations where the substrate can be replenished regularly. The idea of using a bacterium with photoautotrophic and heterotrophic respiration mechanisms is relatively new, and only a few studies have been conducted on the matter (2).

*Rhodospirillum rubrum* is a common purple non-sulfur photosynthetic bacterium that is heavily utilized in the waste treatment process, especially in fertilizer reduction (11, 12). In addition, it is already a target for microbe-driven energy generation due to its ability to generate molecular hydrogen when degrading lactose, a common waste product. Due to its photosynthetic capabilities, its exoelectrogenic properties, and its viability in other major energy sectors, it was hypothesized that this bacterium could be an excellent candidate for novel microbial fuel cell experimentation. Upon research, the team discovered little literature on the capabilities of *R. rubrum* performing in a microbial fuel cell.

The purpose of this project was to show the viability of a *R. rubrum*-powered photosynthetic-microbial fuel cell (pMFC) in experimental laboratory settings as well as real-world situations. It was hypothesized that *R. rubrum* would function in a photosynthetic microbial fuel cell (pMFC) and respond in a predictable manner to a variation of external resistance and wavelength of light exposed to the apparatus.

## Results

Once the prototype was constructed (Figure 2), the next goal was to determine its functionality and take basic relevant measurements of potential and cell growth. To initially test the functionality of the *R. rubrum* cell, a pMFC was left under a fluorescent light for 24 hours, and open potential was measured hourly. Initial testing revealed that *R. rubrum* had a capacity for fuel cell performance, the fuel cell having a maximum open potential of 920 mV upon initial inoculation. To measure the longevity of pMFC's function, an extended run was conducted under the same settings (fluorescent light) over an approximately 168 hour period. The initial potential was 478 mV, and the pMFC only decreased in potential by 100 mV over a 24-hour period, indicating fairly consistent output (Figure 3); from hour 25 to hour 100, the pMFC potential dropped from 355 mV to 339 mV; from hour 101 to hour 167, the pMFC potential dropped to 323 mV. Note that Figure 3 shows only the first 57 hours, since from hour 57-167, there was a total of less than 20 mV drop in voltage. To measure growth, another pMFC was set up and left under fluorescent lighting for a period of approximately 14 days; simultaneously, a culture of *R. rubrum* was maintained in a glass bottle. Concentration of bacteria in both systems was measured at regular intervals using a spectrophotometer. Results demonstrated that *R. rubrum* grew much faster in the pMFC than in a standard bottle, finishing its log phase and entering the stationary phase within 120 hours in comparison to 288 hours, respectively (Figure 4).

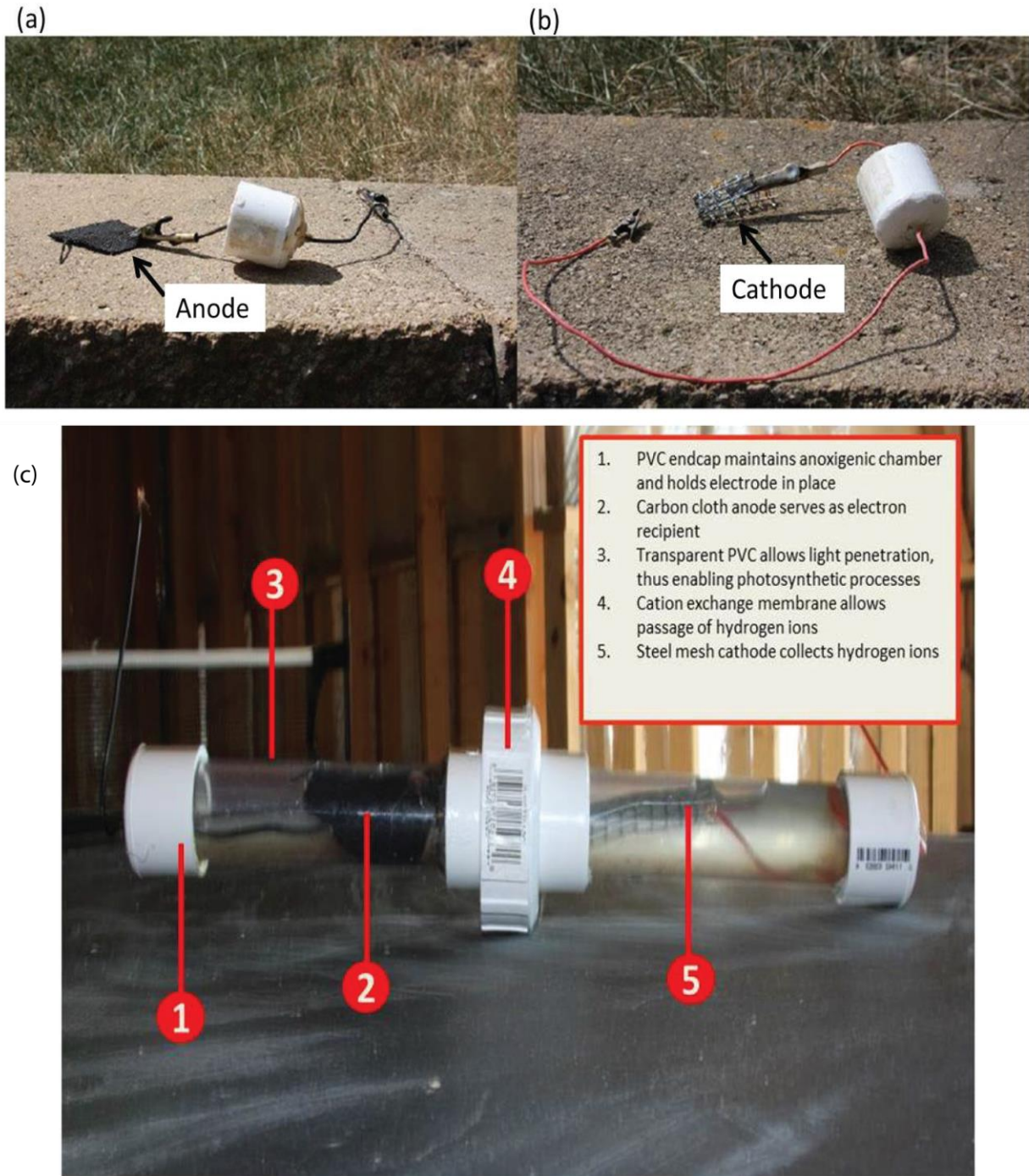


Figure 2: Potential pMFC prototype (constructed). Both electrodes were attached to PVC caps with epoxy and sealant in order to allow connection to main apparatus, which was also constructed from PVC piping. (a) The anode was constructed using a carbon cloth. It was connected to a circuit using an alligator clip. (b) The cathode was constructed using a folded stainless steel mesh. It was connected to

the circuit using an alligator clip. (c) Depicted are the specifications of the final prototype pMFC developed.

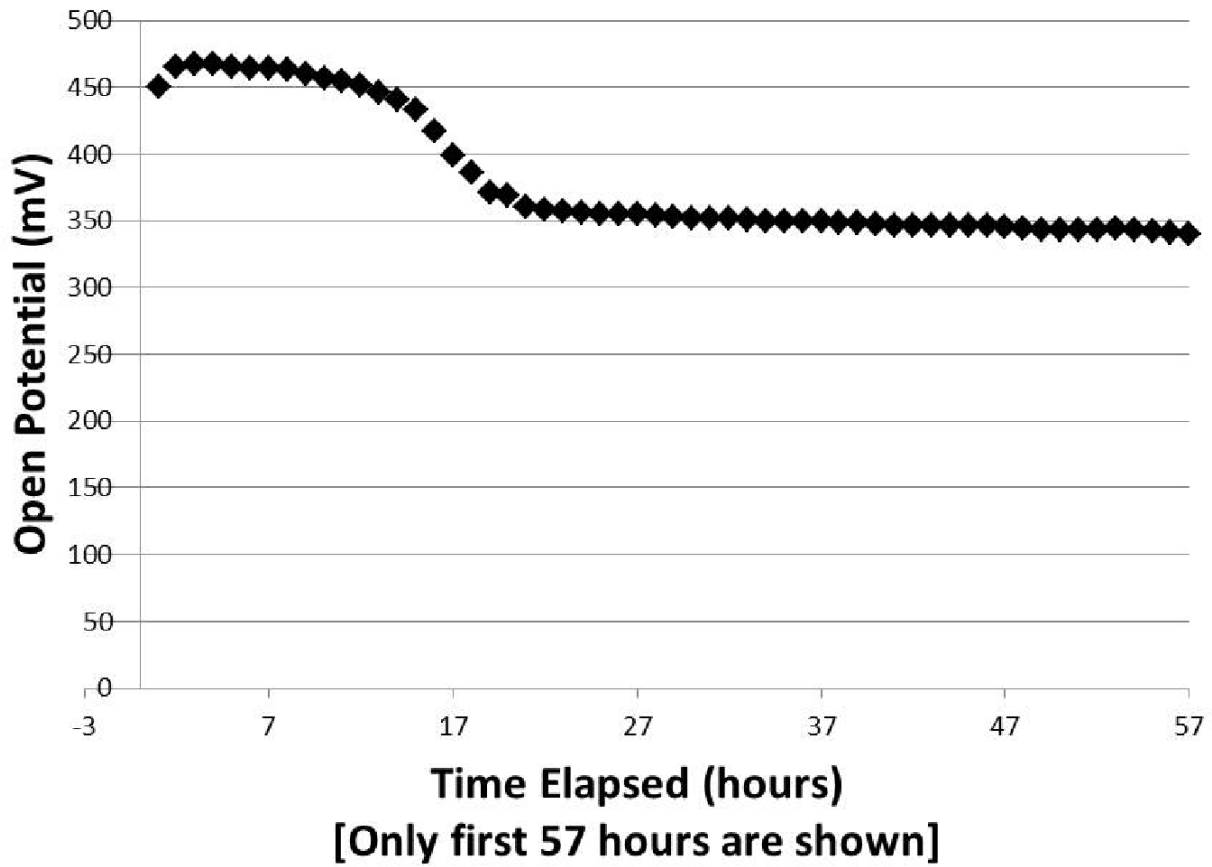


Figure 3: Proof of concept *R. rubrum* pMFC open potential over time. The prototype pMFC open voltage was measured over an extended period of time. The pMFC drops approximately 100 mV within the first 24 hours; note that hours 58 through 167 are not depicted due to the high consistency of the data.

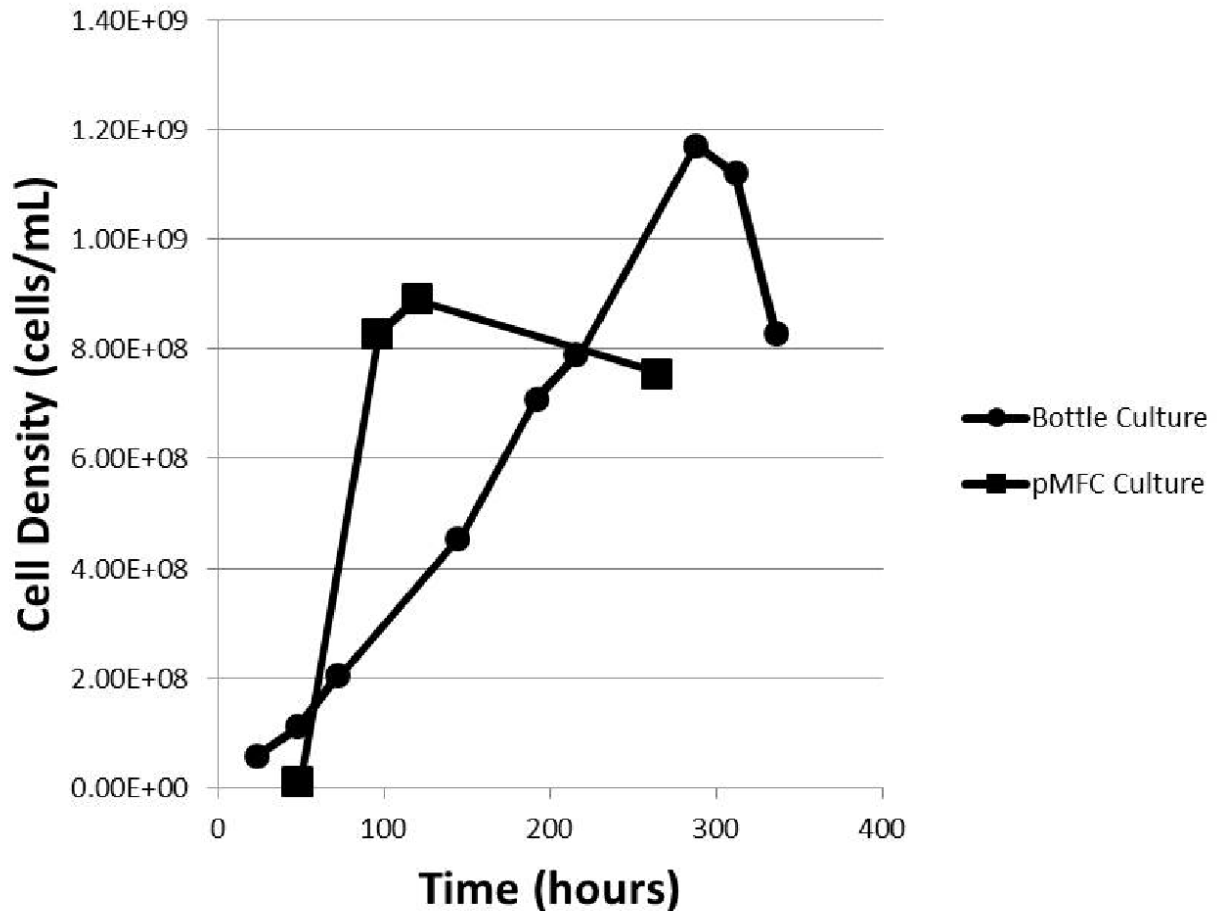


Figure 4: *R. rubrum* growth comparison between bottle and pMFC. The *R. rubrum* cell density was measured at several time points in both a bottle and a pMFC. *R. rubrum* in a bottle culture reaches the end of log phase and beginning of stationary phase at approximately 288 hours. *R. rubrum* in a pMFC culture, in contrast, reaches the end of log phase/beginning of stationary phase within 120 hours.

After confirming functionality, the next goal was to determine optimal operating conditions—namely, to maximize output of the pMFC by modifying wavelength of exposed light. Using a color-changing LED, average power output of sets of 5 pMFCs were monitored over a 24 hour period while exposed to a specific wavelength of light. An ANOVA on the means of the power outputs of the pMFC under different light exposures revealed  $p < 0.0001$ , indicating that the variances in power output did not occur by chance (Figure 5). The peak in measured output occurred under the blue light at  $\lambda \approx 470$ .

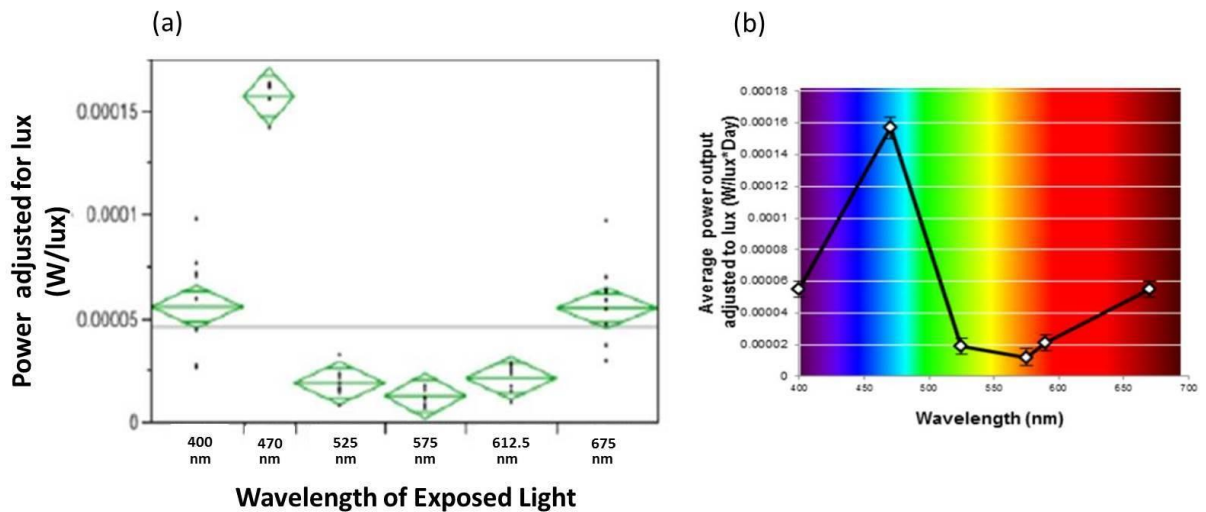


Figure 5: Analysis of differences in power over color. pMFCs were placed under various wavelengths of light using a color-changing LED, and voltage (and by extension power) was measured over a load of 10k $\Omega$ . Lux was also measured using a lux meter and power was then normalized for lux. (a) shows average power outputs for each pMFC plotted per wavelength, with the green diamonds representing 95% confidence intervals. An ANOVA statistical test revealed statistically significant differences between the various wavelength groups ( $p < 0.0001$ ). (b) shows average power output variation plotted against the visible color spectrum. Note that the maximum power output was shown to be found at approximately 470 nm; while this does not correlate to the absorption spectra chlorophyll b or bacteriophyll, it does correlate to the spectra of some carotenoids found in *R. rubrum* (12,13).

While the results so far demonstrated clear effectiveness under fluorescent light, they failed to show the effectiveness of *R. rubrum* under regular sunlight conditions. Thus, a pMFC was maintained in a greenhouse with adequate sun exposure, and both light intensity and voltage were measured over a 10 day period. The *R. rubrum* pMFC performed effectively under the sunlight condition, with only a moderate decline in power output (188 mV) displayed over the course of several days (Figure 6a). In addition, despite lack of a sufficient sample size to perform a t-test, the power tended to increase during the dark periods. A multiple regression analysis of time and light intensity (lux) on power output revealed  $r^2=0.94$  with  $p < 0.0001$ , indicating that 94% of the variation in power output could be attributed to changes in time and light intensity (Figure 6b). The regression breakdown revealed that 77% of the variation in power output could be attributed to the time factor, whereas the other 17% of variation could be attributed to the changes in light intensity.



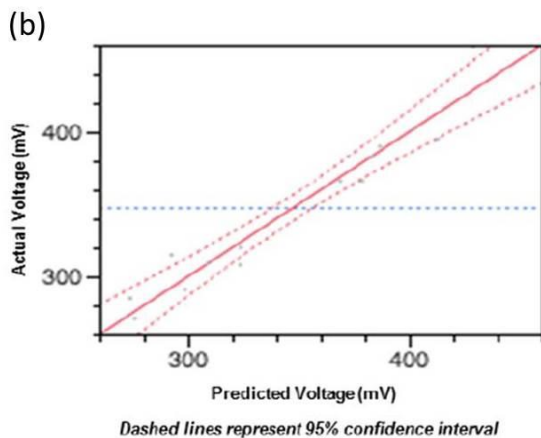
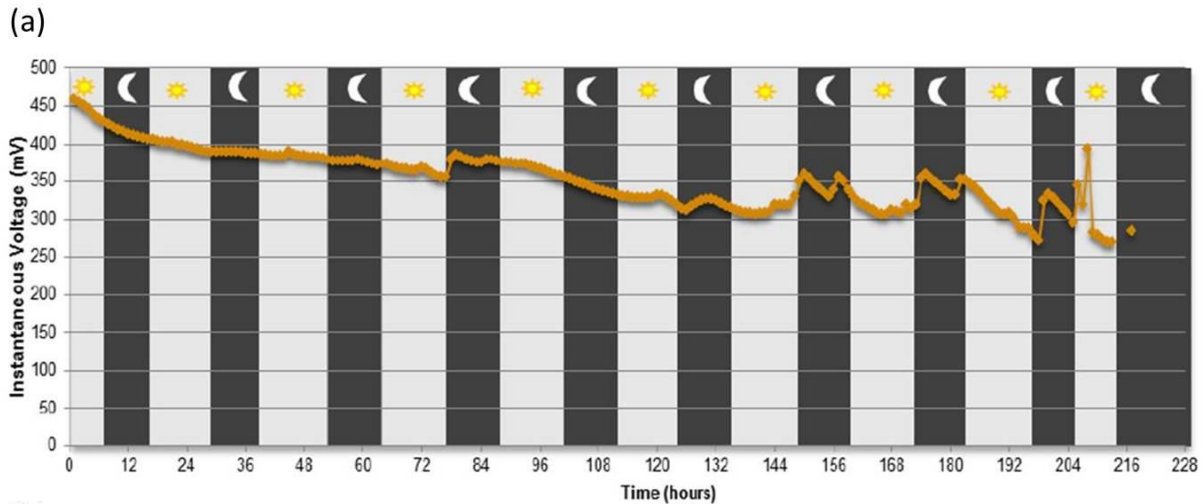


Figure 6: Analysis of pMFC exposed to natural sunlight conditions. A pMFC was kept in a greenhouse which allowed in natural sunlight, and both voltage over a 10 k $\Omega$  resistor and lux (a measure of light intensity) were monitored over the course of several days. (a) depicts the raw voltage data as a function of time and additionally shows day and night periods. The voltage steadily but fairly slowly declined as a function of time, and some voltage peaks were seen in the night periods. (b) depicts a multiple regression analysis of voltage as a function of both time and lux. The results gave an  $r^2=0.94$  with  $p < 0.0001$  and with 77% of variation accounted for in voltage attributed to time and the other 17% of variation accounted for in voltage attributed to lux. The predicted voltage was calculated using the multiple regression model and was plotted against the actual voltage. The dashed lines represent a 95% confidence interval, which when plotted against the actual v predicted line, demonstrates that the multiple regression model is highly accurate.

Finally, an experiment was conducted to optimize load (resistance) of the pMFC by monitoring the power output of a pMFC while modifying resistance using a potentiometer every 30 seconds. The power output of the pMFCs was shown to vary as a result of the modification of external resistance. The

observed maximum output occurred at 295  $\Omega$  resistance. A best fit quadratic model revealed an  $r^2=0.93$ ,  $p < 0.0001$ ; the model predicted an optimized resistance of 231  $\Omega$  (Figure 7).

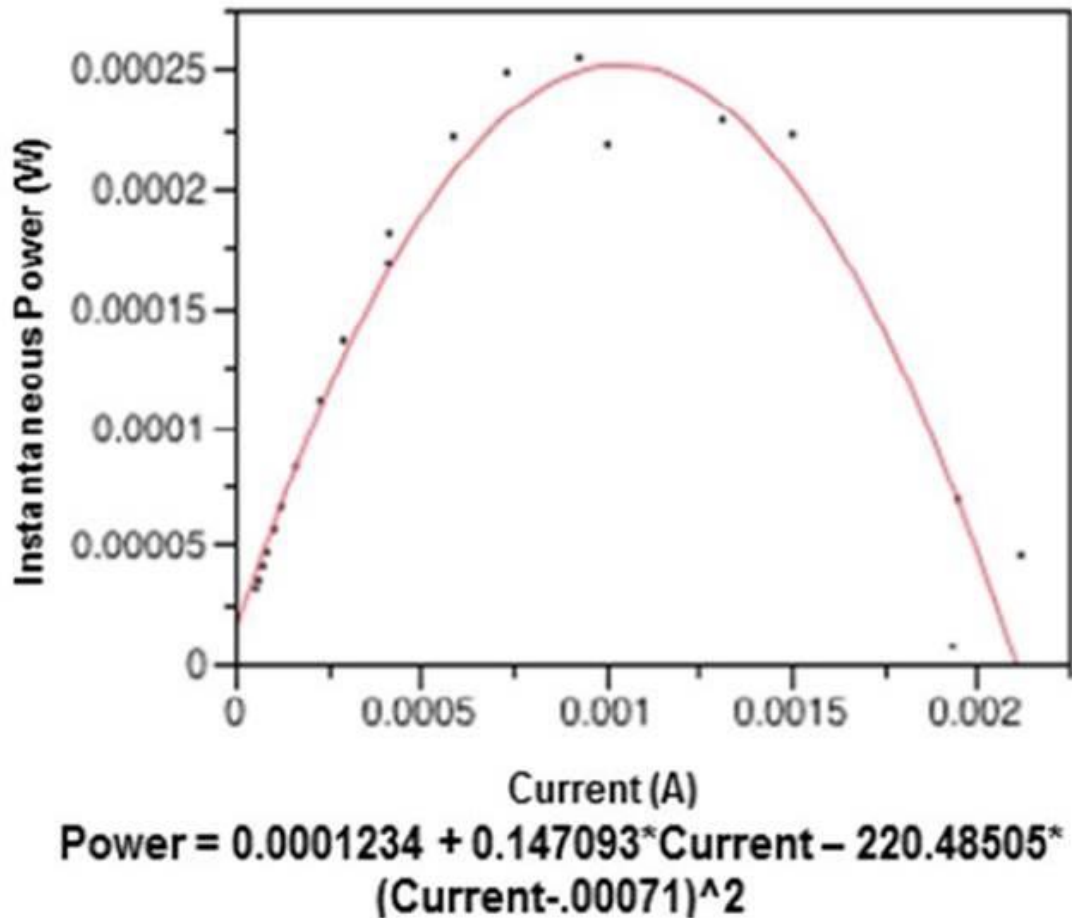


Figure 7: Power curve analysis. In a stabilized pMFC, resistance was modified in 30-second intervals and the corresponding voltage was recorded. Using said voltage data, power and current were both calculated, and power was plotted against current. Using previous research as justification, a quadratic regression was conducted, and a power curve (shown above) was generated (2). The regression had an  $r^2=0.93$  with  $p < 0.0001$ , indicating a statistically significant quadratic relationship between instantaneous power and current; optimal resistance for maximum power output was determined to be 231  $\Omega$  by optimizing the regression model.

## Discussion

In this study, a pMFC was developed with the bacteria *R. rubrum* that could consistently generate power for up to 12 days (limited by measurement). Under the peak output wavelength ( $\lambda \approx 470$ ), the *R. rubrum* pMFC generated  $1.25 \text{ W/m}^2$  (normalized to the anodic area). Interestingly, this wavelength does not correspond with *R. rubrum*'s major absorbance spectrums from chlorophyll b (660-680 nm) and bacteriophylls (800-925 nm) (12); however, some carotenoids shown to be present in *R. rubrum* have absorbance spectra from 460 to 560 nm, a range in which the peak wavelength does fall (13). In comparison to other high power output pMFCs such as an *R. palustris* fuel cell with reported power outputs of  $2.72 \text{ W/m}^2$ , the *R. rubrum* pMFC performed about 44% as effectively (14). A strong multi-faceted relationship was found between time and light intensity, both as functions of power. Spikes in power were seen when the pMFCs were not exposed to light—this spiking is most likely because in the light, cell energy was devoted to both photosynthesis and respiration, whereas in the night, in the absence of light, cell energy was not channeled to the light-phase of photosynthesis and was instead fully channeled to dark-phase and respiration (15). Power curves were also conducted to find the optimal resistance, which was  $231 \Omega$ .

The most viable sector to implement this pMFC technology would be in a wastewater treatment facility, and one could extrapolate the power output in that particular environment. A typical waste-tank treatment center cesspool (diameter  $\approx 20 \text{ m}$ ) can fit an anodic material (such as carbon cloth) with a surface area of  $628 \text{ m}^2$  (16). It has been shown that power density is proportional to the logarithm of anodic surface area; however, not enough data regarding surface area modifications were collected to mathematically justify creating a logarithmic regression model for extrapolation and models for other similar bacteria are not currently available—thus, it is difficult to predict exactly how much power could be generated with a scaled machine (17).

A pMFC could be implemented in a wastewater treatment facility (and other similar facilities including breweries and dairy producers), particularly in the area of fertilizer reduction. The cell would be placed before the secondary clarifier (where the waste is chlorinated), harnessing the power of the microbes constantly being pumped through it. As *R. rubrum* is actively used in this portion of the treatment and reduction process, this proves to be a viable application of the pMFC (12).

In facility layouts that permit it, running MFCs in series and/or parallel may further help to accommodate power output needs. One possible such design would be a stacked pMFC. The system would be made primarily with a Plexiglas shell with alternating anode and cathode chambers. Each anode/cathode layer would be 1 ft tall and have cross-sectional dimensions of 4 ft x 4 ft. One given system of these chambers would have an initial cost of \$1,950 (18, 19). Again, ideally, this price would

drop with increased usage of pMFC technology. Such a system would also be advantageous due to the ease of replacing components such as anodes/cathodes/wires that quickly degrade.

One important aspect to note is that, despite the apparent inefficiency of the *R. rubrum* pMFC in comparison to other pMFCs, the *R. rubrum* pMFC is particularly useful due to its huge presence in the waste treatment process; because of this unique surplus, the results of this study are to some extent more translational than other conventional pMFCs. In an actual application of this pMFC technology, the *R. rubrum* necessary to generate electricity via the fuel cell would already be present in the system (i.e. would not need to be introduced by an outside source).

In general, there are two major areas of interest in terms of pMFC research: the architectural design of the pMFC and the microbial efficiency. In terms of architectural design, after arriving at a decided prototype, all experiments were conducted on the PVC pMFC. However, the architectural factors that may affect power output still have significant areas for improvement. For one, anodic and cathodic materials wear down quickly with use. In order for the pMFC to be a viable alternative, high efficiency materials (i.e. anodes and cathodes that more easily pick up electrons and ions) that have low cost in mass production must be explored. Thus, material degradation would be compensated by the increased efficiency of the electrode materials. Moreover, the surface area of the anode must be considered. Ideally, increasing the surface area of the anode in the same volumetric area would result in an increased overall efficiency; exploring the use of anodic materials such as carbon brushes, which have low volume but high surface area, would be ideal for the pMFC model. Also, cost-effectiveness needs to be considered because, as noted earlier, the cost of implementation of such pMFCs is too high. Probably the most viable area of future interest would be investigating the impact that decreased volume with constant anode/cathode surface area has on power output.

In terms of microbial efficiency, there are two major areas of interest. One concept is to genetically modify *R. rubrum* to increase rate of electron transport/respiration. Ideally, if such a mutation could be identified, the *R. rubrum* could be integrated into pMFC prototypes like the one previously described. The other major route to consider would be exposing the bacteria to either more complex carbohydrates or increased amounts of simple carbohydrates, thereby necessitating higher power output.

It was shown that *R. rubrum* performed effectively in a pMFC, and that the bacteria responded to variations in light wavelength and circuit resistance, allowing the optimization of power output. In addition, the *R. rubrum* pMFC performed effectively under sunlight exposure over an extended period of time, showing the viability of pMFC technology outdoors. Currently, this is the only one of two pMFCs

that have utilized a purple nonsulfur bacteria, the other being a pMFC run by *R. palustris* developed in 2008 (14). Observing the rate of expansion over the past 10 years in terms of research and the power capacities of standard sediment MFCs (2), it is expected that the power capacities of the pMFC will be further researched and optimized for viable modern day use. With the only limiting factor being cost (both for the initial investment and the maintenance of degrading components), it is hoped that, with future studies that venture to increase the viability of pMFC usage, the photosynthetic microbial fuel cell will take its place among the ranks of viable alternative fuels.

## Materials and Methods

A microbial fuel cell is made of two primary chambers: the anode and the cathode. The anode chamber contains a carbon-based electrode upon which the bacteria grow when deprived of oxygen. This electrode is suspended in growth medium and is connected to a wire extending out of the chamber. In order to force the bacteria to transfer electrons to the electrode during the electron transport chain, the anode chamber is kept entirely anoxic. The cathode chamber contains a stainless steel mesh which readily accepts protons (hydrogen ions) suspended in a 0.25 M NaCl solution and is connected to the anode over a resistor. The cathode chamber does not need to be kept anoxic as microbes are not present nor desired in the chamber (see Figure 2a and b for anode and cathode).

Figure 2c depicts the full finalized prototype design. Both chambers were constructed with one foot of 1" diameter PVC piping and were connected using a union. The anode and cathode solutions were separated by a commercially developed Cation Exchange Membrane CMI-7000 (Membranes International Inc, Ringwood, NJ), which is selectively permeable to hydrogen ions. Other substances such as chamber solutions, oxygen, and electrons do not pass through the membrane. A small piece of membrane was placed in the union between the solutions. The electrodes were constructed using carbon cloth for anodes and stainless steel mesh for cathodes (Figure 2a). Electrodes were attached to the end caps of the PVC piping.

Because we are investigating the photosynthetic respiration of *R. rubrum*, clear PVC piping was used, which allowed lights of varying intensities to penetrate the piping and reach the bacteria. For growth media in the fuel cell, a combination of Nutrient Broth Product # 776382 (Carolina Biological Supply Company, Burlington, NC) and Lysogeny Broth with glucose were used.

A 10 mL culture of *R. rubrum* was ordered from Carolina Biological and was transferred into a 125 mL culture of Nutrient Broth using sterile technique and grown for 2-4 days. After the 135 mL culture grew to the constant density (saturated) detected by a SmartSpec® Spectrophotometer (Bio-Rad, Hercules, CA) at  $\lambda \approx 600$ , it was shaken to an even distribution and aliquoted into 5 mL test tubes, which were then

stored at 4°C until needed. On the day of experimentation, the fuel cell chambers were sterilized and the 5 mL culture of bacteria was poured into the fuel cell along with ~70 mL of Lysogeny Broth (enough to fill the entire cell) with a 0.055 M concentration of glucose (or 10 g/L). Each fuel cell was run from 2-14 days, depending on the particular experiment. In addition, five fuel cells were created and run in tandem to minimize experimental error across experiments. During testing sessions, the fuel cell's voltages were recorded via an Arduino® microcontroller (SparkFun Electronics, Boulder, CO) with multiplexer and MATLAB® (The Mathworks, Natick, MA) data collector. Upon completion of experimentation, leftover substrate and all materials used were bleach-treated and disposed of in accordance with BSL-1-standards.

There were four main tests to test the feasibility and performance of an *R. rubrum* -run pMFC. In the first test, a proof of concept, a pMFC with *R. rubrum* was set under controlled conditions, i.e. at 22°C, running under light/dark conditions, 12 hours each, with exposure to fluorescent light to test for *R. rubrum*'s viability as an electron donor. The cell densities of both a bottle culture and the pMFC were measured using a SmartSpec Spectrophotometer at  $\lambda \approx 600$  nm.

To test *R. rubrum*'s response to fluctuations in wavelength, a wavelength test was conducted using a multicolor LED light. Five pMFCs with exact dimensions and features with the same bacterial densities were run in a dark room with exposure for 24 hours to the single LED light source set on specific wavelength. Every 24 hours we changed the wavelength and replaced the anodic and cathodic solutions. Between sets, the pMFCs were disinfected with bleach-water and dried. Experiments were repeated to ensure the accuracy of the results. Power was calculated using the equation:

(Eq 1)

This was then normalized by dividing by lux values as determined by a TES1332 Lux Meter Digital Light Illuminometer (TES Corp, Taipei, Taiwan, and R.O.C).

Thirdly, to measure the viability of an *R. rubrum* pMFC in real-world situations, one *R. rubrum* pMFC cell was placed in a fully exposed greenhouse with an automated multimeter for three days. The light intensity was measured in lux using the TES1332 Lux Meter Digital Light Illuminometer.

Lastly, power curve tests were run on the pMFC. A single *R. rubrum* pMFC cell was used and the experiment modified the resistance every 30 seconds using a potentiometer, and voltage was recorded

using a multimeter. Resistance was varied from 100 – 2000 ohms in 100 ohm increments. Using Ohm's law,

(Eq 2)

the current was calculated at the specific resistance and then substituted into Joule's law,

(Eq 3)

to calculate power.

Voltage data were collected and logged using a Fluke Logging Meter® (Fluke Corporation, Everett, WA) and also an Arduino® microcontroller with multiplexer and MATLAB® (all voltage data, except where noted, were taken over 10,000 Ω). Instantaneous power output was calculated using Ohm's law, and the power output over time was calculated by taking the approximate area over the power data. The analysis was conducted using JMP®9 (SAS Institute, Cary, NC) statistical software.

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