Photosynthetic bacterium for long-term space expeditions

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Abstract

Microbial Fuel Cell (MFC) technology has been pursued since 1911 when Michael C. Potter made his attempt at making electricity using microbes. Now we know that many more microbes can generate electricity in MFC. There are numerous applications for MFC from NASA's standpoint; the largest one dealing with the manned mission to Mars which is expected in 2031. The Mars mission will be an extensive mission with regards to time frame. There will undoubtedly be an accumulation of human waste which has to be dealt with in some way. Per NASA the current proposed solution to the waste generated by the astronauts is to ship the waste back to Earth's orbit, where it can then be intercepted. Another possible application would be similar for the long-term orbit of the International Space Station. However if waste can be turned into electricity this would be of great value to the expedition. We propose to use hydrogen ($H_{2)}$ producing bacterium *Rhodobacter sphaeroides* for a MFC. The purpose of this proposal is to genetically alter a strain of *R. Sphaeroides* to maximize the amount of H_2 produced. We will take hydrogenase genes from another bacterium, *Rhodospirillium rubrum* and introduce them into the *R. sphaeroides* strain. Efficient H_2 production from human waste by *R. Sphaeroides* will help solve accumulation of waste as well as generate electricity on long-term space expeditions.

Background

Microbial Fuel Cells (MFC) are fuel cells that exploit microbial components to generate electricity. Due to the use of microbes there are vast amounts of potential sources. In 1911a electromotoric force with electrodes sitting in bacterial cultures and in a medium, arranged in a battery-like setup, was accomplished by Michael C. Potter (Schröder, 2007). By microbial disintegration of organic compounds, Potter realized that electric energy can be liberated. Later works only confirmed this thought. NASA has been funding MFC since the mid 1960's and as recently as 2004 with a project at the Northwestern University. In the 1960's the idea of MFC was really picked up and researched further by NASA to be applied to space flight. NASA's idea was to transfer waste into electricity by the use of MFC. NASA soon realized that significant time and input would be needed to perfect this system. Research into MFC has been reinvigorated at the end of the 20th century with increased global concern over the use of fossil fuels and climate change. With the manned mission to Mars planned for 2031, NASA is re-evaluating the options for dealing with waste on this long-term 30-month mission. According to NASA a crew of six would generate an estimated 12,000 lbs. of solid organic waste which make MFC a practical option for NASA.

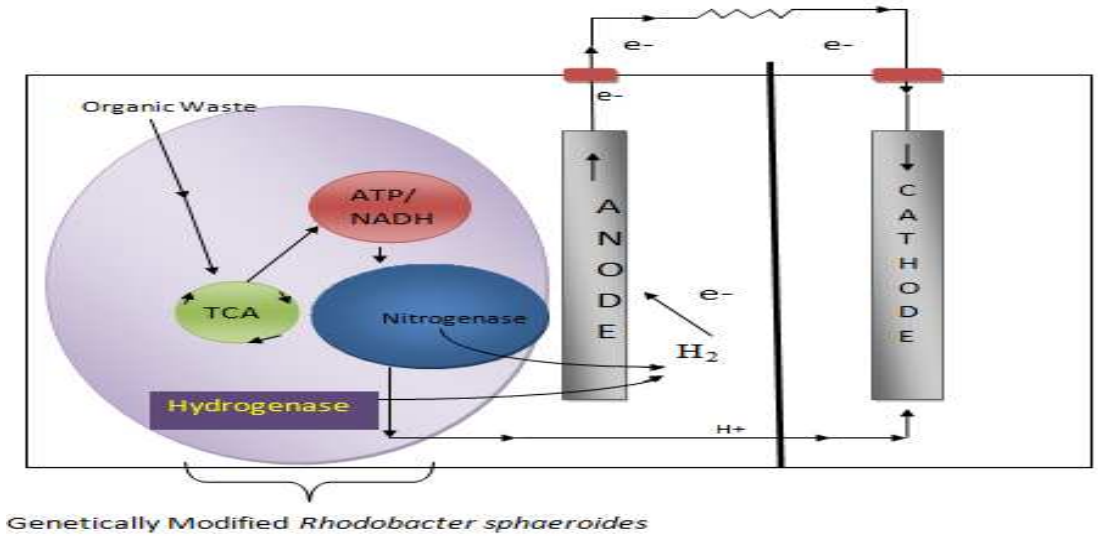

Figure 1 – Proposed Microbial Fuel Cell

There are three components of the MFC. The first is the anode which is responsible for the collection of the electron that has been shed by the metabolism of the microbe. Microbes can produce their electron from organic compounds. The electron charge then travels via a thin wire to the cathode in which provides the other end of the fuel cell on the electron flow. Separating the two is a cation-selective membrane that permits the protons passage. The anode sits in the pure culture of the bacterium and the cathode is submerged in water. In classic MFC, fermentative bacteria grow very sluggish in the waste containing medium that obtains its carbon from that source. The biological and chemical processes lead to an electron being donated to the anode and a proton being communicated via a semi-permeable membrane. By doing this, it creates an electrical charge which is then stored in a battery.

Rhodobacter sphaeroides is a photosynthetic purple nonsulfur bacterium (PNSB). Under anaerobic conditions it produces hydrogen, as long as there is sufficient light for the bacteria. The generation time of *R. sphaeroides is* extremely fast and is one of the fastest candidates for MFC. In recent experiments using a two-stage MFC with *Rhodobacter sphaeroides* Cho and colleagues were able to achieve a power density of 790 mW m^{-2}, which is a very low yield considering the bacterium used (Cho et. al., 2008). The largest power density achieved with a MFC is 5,850 mW m^{-2}, which is clearly an improvement over Cho and colleagues. However with our proposed project, there would be some changes made to this process. *R. sphaeroides* has been extensively studied in our lab and by

Dr. Mark Gomelsky. The reason we have chosen to work with *R. sphaeroides* for this project is due to the fast generation time compared to a fermentative bacterium, as well as the plentiful abundance of light energy derived from the sun. Currently in Dr. Gomelsky's lab there are experiments with regards to a mutated strain of *R. sphaeroides* in an attempt to increase the H_2 yield (Gomelsky et. al., 2004). The metabolism of *R. sphaeroides* starts with organic acids; derived from human waste; as the carbon source to induce the Krebs cycle and produces NADH. *R. sphaeroides* produces large amounts of energy/ATP via NADH by photosynthesis. Then NADH and ATP are used in nitrogenase which produces the electrons and protons needed by the anode and cathode of the MFC (Figure 1). Note that since this is done in the absence of N_2 the reductions from nitrogenase produces H_2. An added benefit is that once a biomass of *R. sphaeroides* has accumulated on the anode of the MFC, it has a secondary use as a nutritional supplement for aquatic animals, fish in particular. Given the proposed application for space expeditions, there could be use of this with any sort of aquatic farms used as a food source for crew (Yamaoka et al., 2008).

A photosynthetic PNSB *Rhodospirillium rubrum* uses CO as its carbon source and produces copious amounts of H_2 as a byproduct using the membrane-bound hydrogenase. One of the goals would be to take the genes in *Rhodospirillium rubrum* of hydrogenase enzymes that control the yield of H_2 and integrate them in the *R. sphaeroides*. Since *R. sphaeroides* doesn't have H_2 producing hydrogenase, inputting the genes responsible for this in *R. sphaeroides* is expected to increase the yield of H_2 in the application of the MFC.

Goals and Specific Aims

The long-term goal is to generate a strain of *R. sphaeroides* with a maximum H_2 yield that would be an efficient bacterium to be used in a MFC. The aim of my proposal is to achieve a high yield, more than 5,850 mW m^{-2}, bacterium that can be inputted into an MFC for use on long-term manned missions as well as long-term orbits to alleviate the waste issue and generate electricity.

Experimental Design

1. Clone hydrogenase from *R. rubrum*.

The hydrogenase in *R. rubrum* is large at 11.0 kb and due to its size; amplification via PCR is not efficient or accurate enough for this particular application. Primers will be designed that are complimentary to the nucleotide sequences at the 5' end of *hydrogenase*-coo*MKLXUH* (Figure 2a). The PCR amplified fragment will be inserted into the suicide vector (pLO1) which contains a kanamycin antibiotic resistant marker (Figure 2b).

The recombinant plasmid will be mobilized into *R. rubrum* and integrated into the 5'end of the hydrogenase genome by crossover recombination (Figure 2c). The mutant *R. rubrum* will be selected by the antibiotic resistant marker aforementioned. We will then digest the hydrogenase complex connected with the antibiotic resistant marker in *R. rubrum* with appropriate restriction enzymes (Figure 2d). The digested complex will be inserted into vector (pRK415), containing a tetracycline antibiotic resistant marker (Figure 2e). The plasmid will then be selected from the medium containing kanamycin and tetracycline.

2. Mobilization of cloned hydrogenase into *R. sphaeroides.*

The construct will be mobilized into *R. sphaeroides* by conjugation and selected from a medium containing tetracycline (Figure 2f).

3. Compare level of H_2 production from strain containing hydrogenase to wild type *R. sphaeroides* 2.4.1.

H_2 production will be measured using gas chromatography with the genetically altered *R. sphaeroides* containing *R. rubrum* hydrogenase. A comparison will be analyzed between the wild type of *R. sphaeroides* 2.4.1 and *R. sphaeroides* harboring hydrogenase.

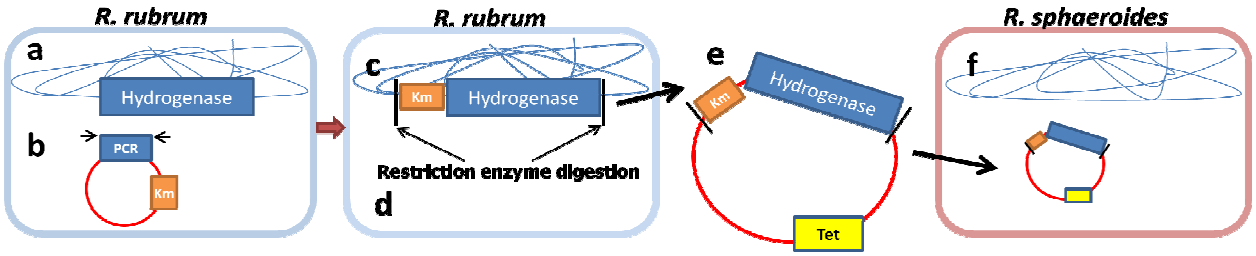

Figure 2-Cloning Strategy

Role and Responsibility of the Student

As the student my first goal will be to follow my mapped out experimental design and account for any interference with the project or unforeseen delays. My responsibility will be to become further accustomed to the numerous tools at my disposal. I have been working in Dr. Gomelsky's lab since the fall 2008 semester and have already partaken in multiple projects and have had hands on experience with real-time PCR, constructing primers, complementation of DNA and DNA purification. For guidance, I will have Min-Hyung Ryu; a current PhD student at University of Wyoming; as well as Associate Professor Mark Gomelsky. It will also be my responsibility to write a report of my findings to the Wyoming NASA Space Grant Consortium, create a PowerPoint presentation, write a scientific article for submittal to a peer-reviewed journal as well as write a general audience article for publication with one of the University of Wyoming publications.

Timeline

Starting in May of 2009 a total of 14.5 weeks will be spent on this project. It will take approximately seven weeks to clone a hydrogenase gene cluster from *Rhodospirillium rubrum*. Then approximately four weeks to transfer the cluster into *Rhodobacter sphaeroides* mutant optimized for H_2 production. The remaining 3.5 weeks will be spent on testing the functionality of *R. sphaeroides* with regards to H_2 production as well as troubleshooting any issues that may have arisen in the course of the experiment. Also during the last phase of the experiment I will spend time complying with the contingent guidelines, writing a report to NASA, making a PowerPoint presentation for Undergraduate Research Day and help write a peer-reviewed scientific journal. Finally I will write and submit for publication an article for general audiences on the topic of this project for one of the University of Wyoming publications (i.e., Branding Iron, Agademics, Agricultural News or Reflections).

References:

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3. Gomelsky, M., et al. Construction and validation of the Rhodobacter sphaeroides 2.4.1 DNA microarray: transcriptome flexibility at diverse growth modes. J Bacteriol 186, 4748-58(2004).
4. Schröder, U. Anodic electron transfer mechanisms in microbial fuel cells and their energy efficiency. Phys Chem Chem Phys 9, 2619-29(2007).
5. Yamaoka, Y., et al. Isolation of a Thermotolerant Photosynthetic Bacterium, Rhodobacter sphaeroides Strain, NAT, and Its Capacity for Oil and Chemical Oxygen Demand Removal at High Temperatures. Biosci., Biotech., and Biochem. 72, 1601-03(2008).

Award Request

The requested award for this fellowship would be $5,000.00 at $8.75 per hour, 40-hours per week for a total of 14.5 weeks. The $5,000.00 will be used as the salary for me during the summer semester. There will be matching funds of $4,000.00 for the advisor and PI's summer salary as well as approximately $1,000.00 in funds from a NSF grant which will be used for supplies.

Budget

#	Category	Amount	Source	Matching Funds
1	Student Salary	$5,000.00	NASA	
2	PI's Summer Salary	$4,000.00		NSF
3	Supplies	$1,000.00		NSF