National Aeronautics and Space Administration

Algae and Cyanobacteria Behavior with Fixed Variables for Space Missions

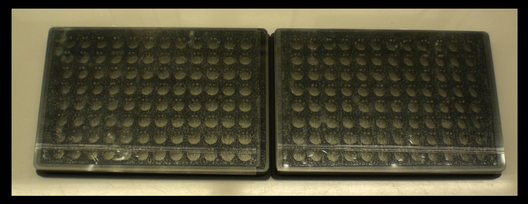
Leona Wong, Brad Bebout, Erich Fleming

Introduction

The study of photosynthesis for nutrient and oxygen cycling in closed systems is vital for the future of human space explorations, as they must be self-sustainable for long durations of time. Algae and cyanobacteria are the most basic organisms known to perform photosynthesis and are also potential food sources, so it is important to explore their behavior in the conditions of space. Growth rate and photophysiological changes will be measured Pulse Amplitude Modulated (PAM) fluorescence. In order to choose the best suited candidates, different strains of algae will be grown in varied conditions, which is the area of focus in this study.

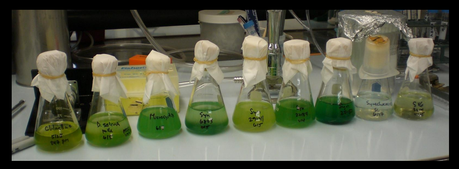
Method

Two identical 96-well plates will be grown with different intervals of light exposure. Plate 1 will be exposed to a cycle of 15 minutes in light, followed by a 30 minute dark period. Plate 2 will also be exposed to 15 minutes of light, but will be followed by a 60 minute dark period.



Both plates will have 10 wells each of different types of algae and cyanobacteria:

Sample 1: Chlorella V.
Sample 2: D. Salina (polle)
Sample 3: Microcystis
Sample 4: Synechococcus 6803
Sample 5: Synechococcus 29141
Sample 6: Synechococcus 27184
Sample 7: Synechococcus 27150
Sample 8: Synechococcus SL
Sample 9: Scenedesmus SV6



Optical density measurements will be taken twice daily – once in the morning and once in the afternoon. This will measure the growth of the organisms.

PAM fluorometry measurements will be taken once a day during a dark cycle. Measuring the photosynthetic efficiency will indicate fitness.

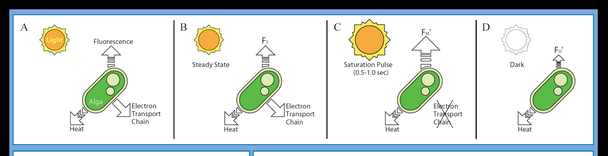


Figure 2. PAM fluorometry schematic. A. Light energy absorbed by algae is processes in three ways: 1) it is used to excite electrons that are then passed down the electron transport chain 2) it is released as heat or through "non-productive" pathways or 3) it is released as fluorescence. PAM fluorometers measure the fluorescence of an algal sample at any given time (B). To determine photosynthetic efficiency (i.e. fitness) the fluorometers generates an intense pulse of light, effectively saturating the electron transport chain wherein all excess energy is released as fluorescence (C). The relative difference between Fs and Fm' [(Fm' -Fs)/Fm'] represents the efficiency of the algae to move electrons down its electron transport chain. Short transitions of algae into the dark (D) allow for the measurement of Fo' or the minimum fluorescence of light-adapted algae. When algal samples are allowed to adapt to dark conditions (>15 min), the values illustrated in B, C become Fo and FMdark, respectively.

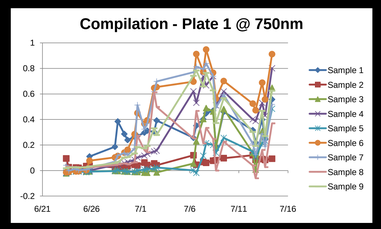
Table 1. PAM Fluorometry parameters

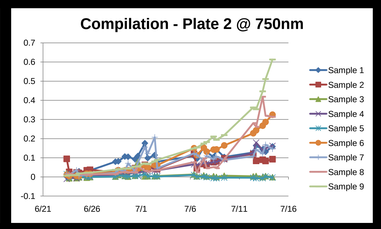
Fluorescence	Equation	Description
F_S		
F_M'		Steady-state fluorescence of a light-adapted sample
F_O'		Maximum fluorescence of a light-adapted sample
F_M		Minimum fluorescence of a light-adapted sample
F_O		Maximum fluorescence (DCMU treatment)
F_{MdarK}		Minimal fluorescence of a dark-adapted sample
		Maximum fluorescence of a dark-adapted sample
F_V'	$F_M' - F_O'$	Maximum variable fluorescence
$\Phi PSII$	$(F_M' - F_S)/F_M'$	Quantum yield of PSII
F_V'/F_M'	$(F_M' - F_O')/F_M'$	Maximum quantum yield of PSII
qP	$(F_M' - F_S)/(F_M' - F_O')$	Photochemical quenching or percentage of open PSII centers
qN	$1 - F_V'/[F_V/F_O)+F_O]$	Nonphotochemical quenching

Data

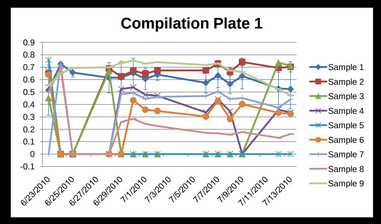
Photographs of Plate 1 (left) and Plate 2 (right), Day 20

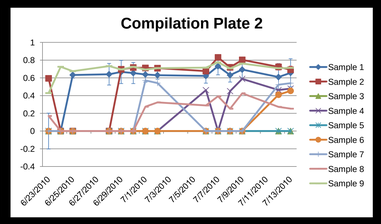
Optical Density





PAM Fluorometry





Analysis

Sample 1: The optical density growth curve from Plate 1 shows relatively quick growth and then a sudden crash -- most likely due to oxygen bubble formations hindering clear measurements. The growth in Plate 2 is gradual and relatively slow. PAM measurements show that both plates are maintaining good health. Also note that a few of the wells are not growing at all and some are doing well.
Sample 2: Shows similar data to Sample 1, but all the wells are growing.
Sample 3: Data indicates that there has been no growth.
Sample 4: Shows similar data to Sample 2.
Sample 5: Data indicates that there has been no growth.
Sample 6: Optical density data shows quick growth in Plate 1 with formation of a lot of gas. The data from Plate 2 is similar to other slow-growing samples. The PAM data shows a maintained level of photosynthetic efficiency.
Sample 7: Shows similar data to Sample 6.
Sample 8: Shows similar data to Sample 6, but with even a faster formation of gas bubbles.
Sample 9: Optical density data shows that the growth is slightly slower in both plates and also a slower formation of gas. The PAM data indicates higher photosynthetic efficiency levels compared to the other samples.

Results

In comparing the data from the two plates grown in different durations of light exposure, it is clear that the conditions of Plate 2 are more viable for longer sustained growth and study. More importantly, this study helped to narrow down certain algal and cyanobacterial cultures for more in depth study.
The collected data and analysis shows that Samples 1, 2, 4, and 9 may be good candidates for further study because they showed relatively slow growth, slow formation of gas bubbles, and maintained photosynthetic efficiency.

Conclusion

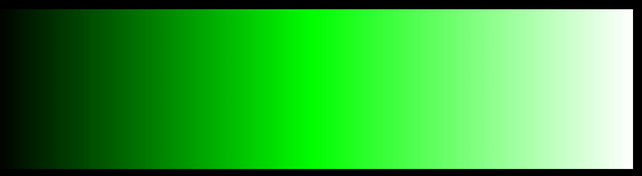
The next step for this study is to design another experiment to figure out if the different cultures can be grown in even longer dark cycles and how long those dark cycles could be. Because of the eventual goal of studying these organisms in space, they will have to endure very long dark periods of travel time. These cultures will have to be able to grow slowly and produce minimal bubbles for the best possible results.

Education Connection

A “color wheel” that relates optical density and the various shades of green found in algae and cyanobacteria will be made for affordable classroom use. Many schools are unable to pay for expensive pieces of science equipment, but that should not deter the children’s education. A successful completion of this project will enable classrooms to have approximate optical density readings just by visual observation or by taking a picture of their specimens and uploading them onto their computers, instead of using a spectrophotometer.

Choosing a few commonly studied organisms, we will observe and record their colors, shade, and optical density readings for various dilutions of the cultures. With enough data, we will be able to construct and test the “color wheel.”

A gradient from black to green to white that may be used to match with algae and cyanobacteria colors.



This project is still at its beginning stages, but with continued study and the collaboration of scientists and teachers, this may soon be a low-cost tool to be widely used in many classrooms.

www.nasa.gov