Data from Raceway Experiments

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In this study, *S. platensis* was cultivated in three open-channel shallow artificial ponds called raceway ponds. Samples of the raceways were collected to measure pH, optical density, oxygen concentration, chlorophyl *a*, salinity, and PAM measurements throughout the *S. platensis* growth cycle. Water and air temperature, pH, irradiance measurements were continuously taken in an automated way.

Figure 1 Experimental raceway

Introduction

S*pirulina platensis* is a filamentous cyanobacterium, consisting of blue-green filaments of cylindrical cells (1 to 12 μm diameter) in unbranched helicoidal trichomes. The filaments are motile, gliding along their axis, and have no heterocysts. *Spirulina* [1] can colonize in environments that are unsuitable for many other organisms, forming populations in freshwater and brackish lakes and some marine environments, mainly alkaline saline lakes [2].
For the past two decades, *S. platensis* has been a focus of interest among researchers in various fields because of its commercial importance as a source of protein, vitamins, essential amino acids, and fatty acids. It is being developed as the "food of the future" because of its amazing ability to synthesize high-quality concentrated food more efficiently than any other algae. Most notably, *S. platensis* is more than 50 percent complete protein, with all essential amino acids in perfect balance. It has a photosynthetic conversion rate of 8 to 10 percent, compared to only 3 percent in such land-growing plants as soybeans [3].
Outdoor cultivation in open raceway ponds is the most commonly employed process for commercial algal production which attempts to maximize the conversion of solar radiant energy and inorganic chemicals into high-value products [4]. There are some drawbacks to open pond cultivation. It is difficult to maintain monoalgal cultures, optimal population density and optimum temperature. Supersaturating light intensities result in photoinhibition and high dissolved oxygen levels. These factors which affect productivity have led to high operational costs or closure of several commercial ventures. Innovations that enhance biomass productivity will facilitate wider application of microalgal products and processes [5].
NASA's renewed commitment to human exploration emphasizes technologies and capabilities enabling human exploration. Of particular importance is the acquisition of O_2 for life support and propellant for ascent vehicles. The diverse metabolic capabilities of microalgae make them key targets for development and integration into ISRU and life-support systems of O_2 production and/or CO_2 sequestration in many exploration scenarios. It is thus critical to NASA's long term goals to understand how these organisms perform on Earth and space environment.
In this study, *S. platensis* was inoculated in three open raceway ponds throughout its growth cycle. Several variables were studied in detail to determine which would be most beneficial for a successful growth cycle of *S. platensis.* We expected to witness and analyze data of an entire bacterial growth of *S. platensis*, including lag, exponential, stationery, and death phases. Figure 1illustrates this growth cycle.

Materials and Methods

The cyanobacterium *S. platensis* 29408 was obtained from ATCC. This strain was isolated from a saline marsh, in Del Mar Slough, CA. Three raceways were constructed and operated in a rooftop greenhouse. The raceway ponds have a 200 liter capacity and a paddle wheel with four blades that rotates at a speed of 6.785 rpm. All raceways were inoculated with 1.5 liters of S. platensis culture and 200 L of *S. platensis* media. Automated and manual measurements were performed from the three raceways throughout the *S. platensis* harvesting cycle. Automated measurements of for temperature and irradiance were downloaded from a datalogger weekly. Manual measurements of pH, salinity, oxygen concentration, nutrients, dissolved inorganic carbons, chlorophyll a, and optical density were performed every day at the same time. Air temperature was the only controlled factor throughout the duration of the experiment.

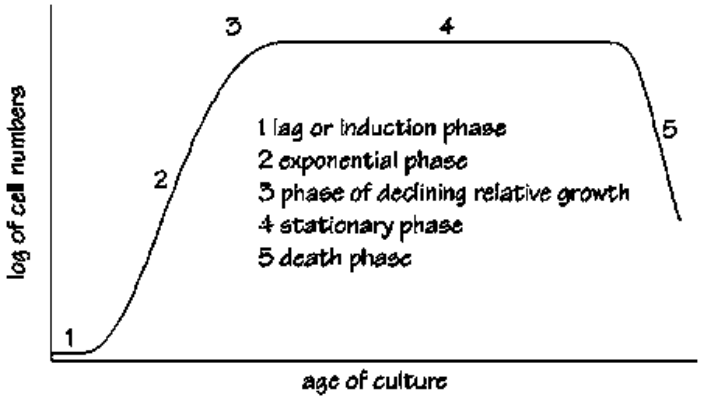

Figure 2 - Bacteria Growth Cycle

Data obtained from the three raceways were obtained using different sensors and taking samples periodically. The data were collected with different timing (temperature and irradiance sensors take measures every 5 minutes, while all the other data are collected, most times, 3 times per week). For that reason, resampling in a daily basis and filtering of the data for removing the measurement noise was performed for displaying the data.

A. Temperature

Temperature values were continuously taken in an automated way. Temperature data (measured in ^{o}C) were collected using Hobo data loggers floating in each raceway. Data were downloaded from the data logger every week.

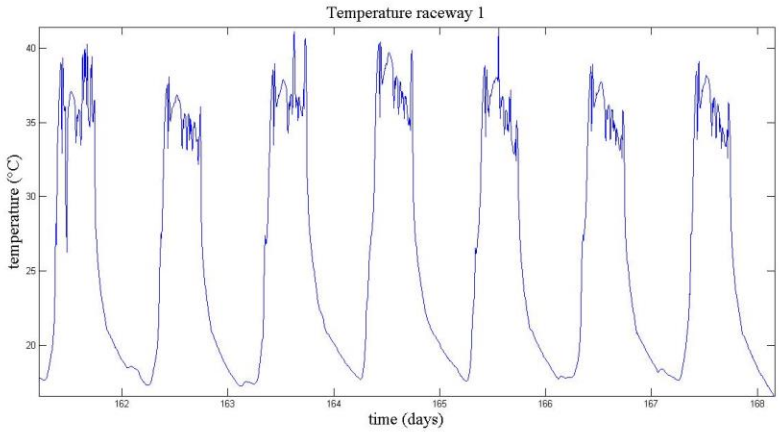

Figure 3 - Temperature

B. **Irradiance**

Irradiance values were also continuously taken in an automated way. Irradiance data (measured in W/m^2) were collected and stored using a Li-Cor datalogger couple to a cosine corrected quantum sensor. Data were downloaded from the data logger every week.

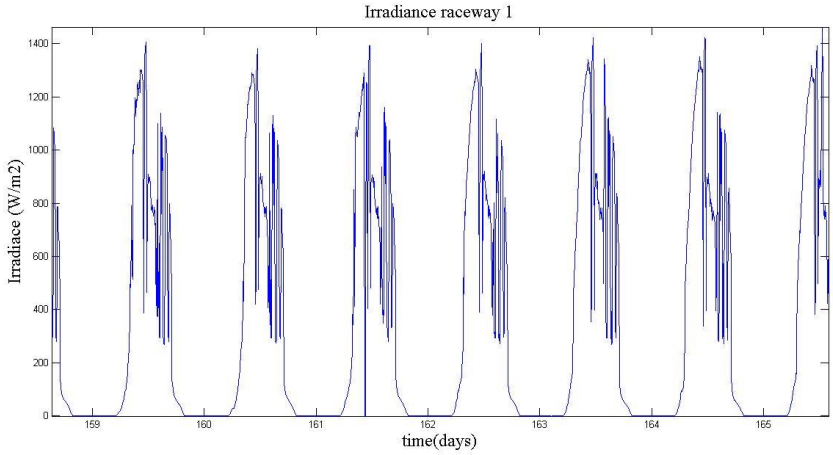

Figure 4 - Irradiance

C. **Optical Density**

Culture density and growth rate were estimated by measuring optical density (OD) using a Shimadzu UV Spectrophotometer. OD measurements were set at 750 nm using 1mL of sample in a glass cuvette. Dilutions were performed when samples became too dense for measurements. Absorbance readings were recorded. Optical density is the logarithmic ratio of the radiation falling upon a material, to the radiation transmitted through a material. When dilutions were performed, the dilution factor was multiplied by the absorbance value.

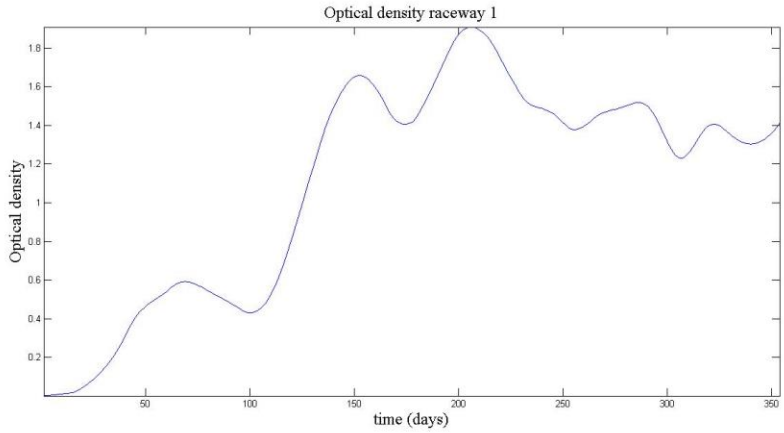

Figure 5 – Optical Density measurements

D. **pH**

pH, a critical parameter for growth rate of algae, was monitored and measured daily. Samples of 25 mL from each raceway were collected in 50 mL falcon tubes. Shaken well, pH measurements were made from each falcon tube with a calibrated Beckman digital pH meter.

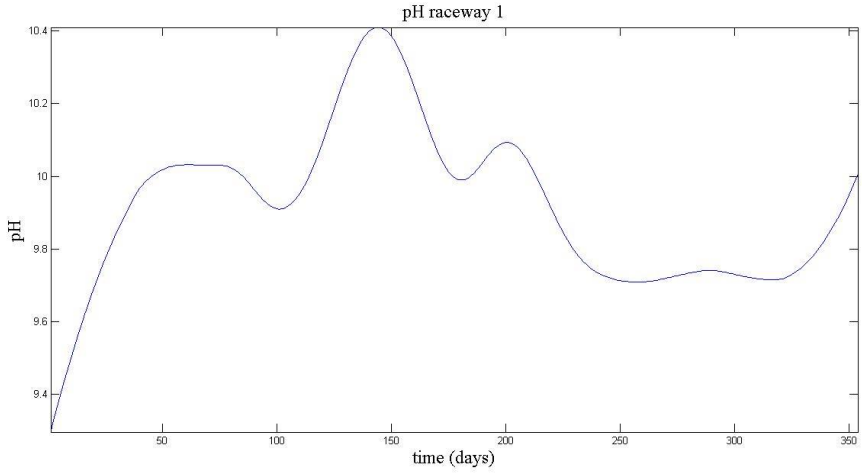

Figure 6 – pH measurements

E. **Nutrient Analysis (Nitrates and Phosphates)**

Macronutrients for *S. platensis* are generally considered to be nitrogen, phosphorus, and silicon. However, silicon is required only for diatoms, silicoflagellates, and some chrysophytes. These macronutrients are generally required in a ratio of 16N:16Si:1P. According to the Redfield ratio, the chemical composition for the average phytoplankter is 106C: 16N: 1P [6].
From the 25mL of daily sample collected from each raceway, 10mL was centrifuged for 10 minutes in a 15 mL Falcon tube on speed 9 G. The supernatant was decanted and store in a 30mL scintillation vial. The vials were stored in a -20°freezer until ready for weekly nitrate and phosphate analysis. 500 µl of nutrient sample from scintillation vials analyses were run on a Dionex Ion Chromatograph DX-120 following its protocol.

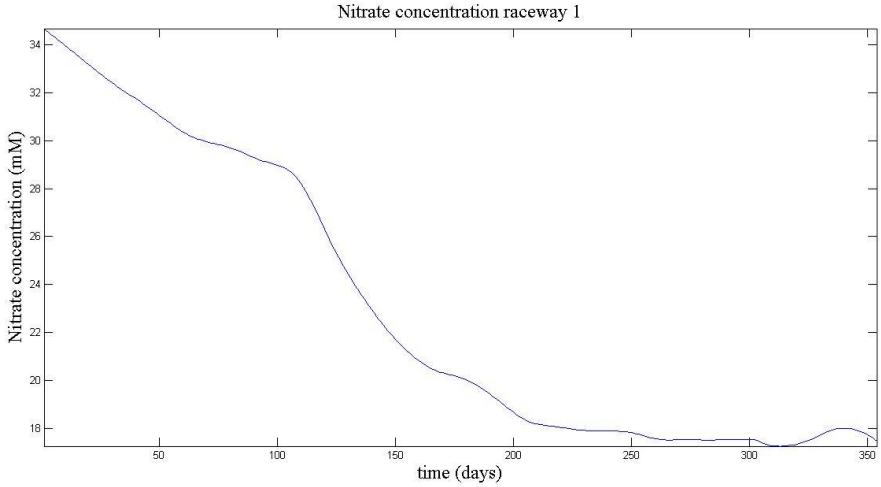

Figure 7 – Nitrate measurements [mM]

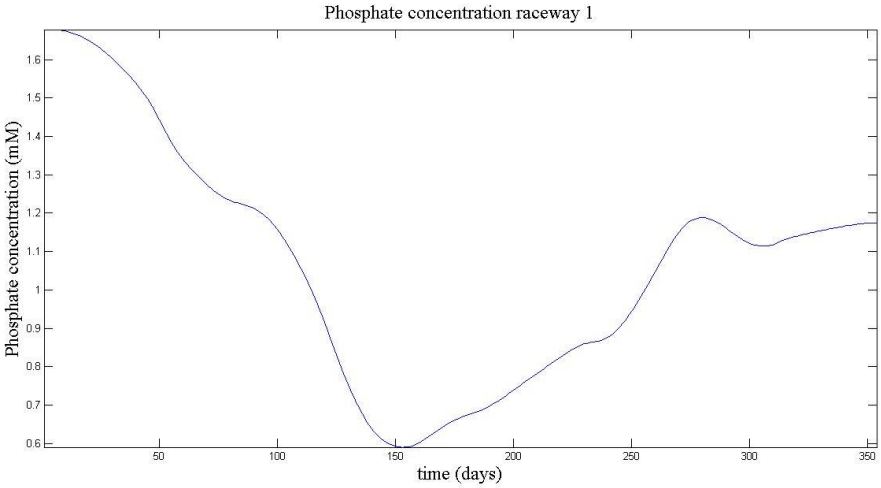

Figure 8 – Phosphate measurements [mM]

F. **Chlorophyll *a***

Chlorophyll *a* is the green pigment that allows plants (including algae) to convert sunlight into organic compounds during photosynthesis. Of the several kinds of chlorophyll, chlorophyll *a* is the predominant type found in algae. Chlorophyll *a* absorbs well at a wavelength of about 400-450 nm and at 650-700 nm. It is ubiquitous in microalgae and the methods for measuring it are sensitive, precise, and mostly accurate [7].
Initially, the10 mL pellet from nutrient analysis was removed and 1mL of methanol with $MgCO_3$ was added to the pellet to extract chlorophyll *a*. This solution was then placed in an eppendorf tube and centrifuged for 5 minutes at 16 rcf (relative centrifugal force). When needed, dilutions were performed to fully extract all chlorophyll *a*.
The eppendorf tubes were stored overnight in a 4°C refrigerator covered with aluminum foil to be analyzed the following day. Measurements of absorbance at wavelengths 665 nm and 750 nm for chlorophyll *a* were taken using a UV-1800 Shimadzu UV Spectrophotometer.
It should be noted that chlorophyll measurements were degraded towards the end of the experiment.

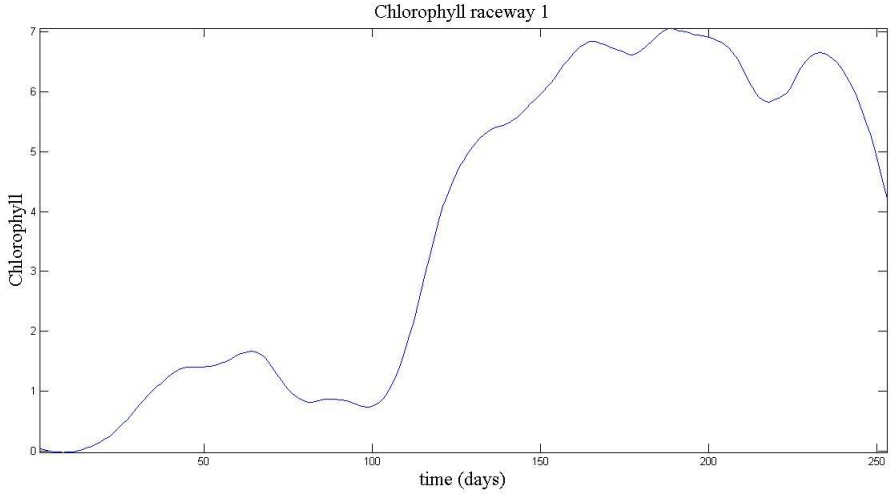

Figure 9 – Chlorophyll measurements

G. **Oxygen Concentration**

Oxygen concentrations were measured by collecting 50mL of sample from each raceway. A Clark-style oxygen microelectrode coupled to a Unisense picoammeter was lowered into each tube and the current of the electrode was recorded. To calibrate the electrode, a two point calibration was performed; two samples were bubbled with nitrogen and air for approximately three minutes and the current of the electrode was recorded. The number of micromoles of oxygen at air saturation was obtained from standard tables, the number of micromoles of oxygen in the nitrogen bubbled standard was assumed to be zero. Measurements were in µmol/L

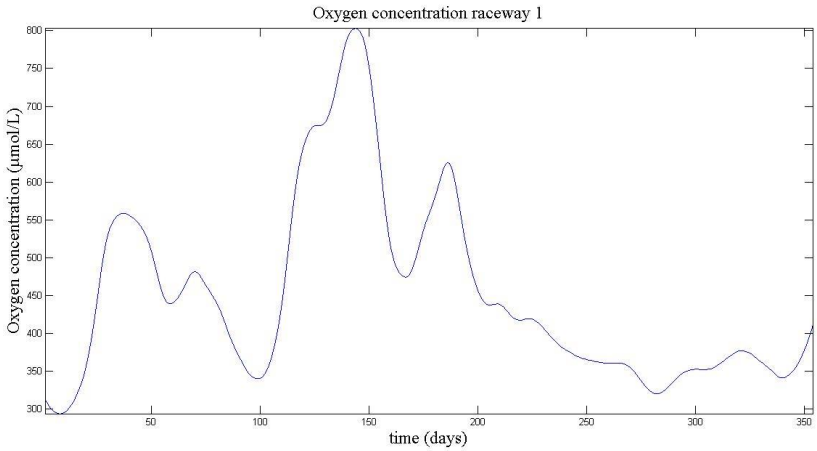

Figure 10 – Oxygen measurements

H. **Salinity**

Salinity is the amount of salts dissolved in a quantity of water. In oceanography, it has been traditional to express salinity as parts per thousand, which are approximately grams of salt per kilogram of solution. Salinity is an ecological factor of considerable importance, influencing the types of organisms that live in a body of water. *S. platensis* experiences optimal growth in a salinity of 10ppt, ideal to the water in which it thrives. A refractometer was used to measure the salinity of each raceway.

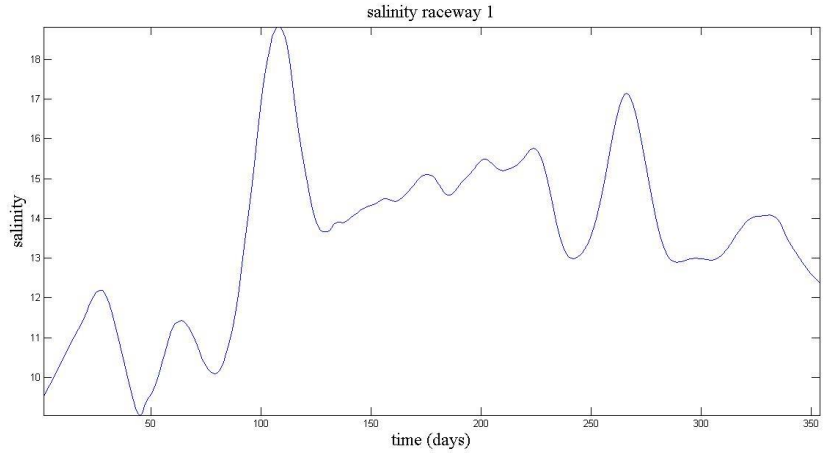

Figure 11 – Salinity measurements [ppt]

I. Pulse Amplitude Modulated (PAM) Fluorometry

PAM fluorometry measures various aspects of photosynthetic metabolism based on the natural fluorescence of the light energy absorbing pigment chlorophyll *a.* PAM fluorometry provides information about the general condition of *S. platensis* in the raceway ponds, key to the well-being of photosynthetic organism and a very sensitive indicator of stress.

The most straight-forward PAM measurements are that of photosynthetic efficiency, derived using the saturation pulse method, and defined as "photosynthetic yield." Photosynthetic yield quantifies the proportion of captured photons that are converted to useful chemical energy. Light energy absorbed by the photosynthetic apparatus (specially the oxygen splitting complex PSII) has three possible fates: 1) generation of photochemical work, electrons stripped from the water reduce electron acceptors in the electron transport chain, 2) heat generation with loss of energy, and 3) energy dissipation by fluorescence, which can be an important shunt for excess energy in order to prevent damage to the photosystem. During a measurement of photosynthetic yield, a very bright "saturating pulse" of light-typically blue or white-is directed at the sample. During that pulse,, the capacity of the electron transport chain to accept electrons from the photosystem saturates and all the surplus is dissipated as fluorescence, which rises to a maximum (known as "maximal fluorescence" F_m), the plateaus (the energy lost to heat is unchanged during this relatively short period of time). The change in fluorescence between F_t, and F_m is called the "variable fluorescence" (F_v). Photosynthetic yield is defined as the variable fluorescence normalized to the maximum fluorescence (F_v/F_m).

In this study, PAM measurements were taken using a Walz PAM fluorometer (Diving PAM) everyday when irradiance was highest, usually at the 12:00 hr. Measurements were taken at three depths in each raceway, depending on the depth of the raceway for that day. The raceways ponds typically had a depth of 16 cm; therefore surface measurements were taken at 0 cm. Midwater measurements were taken at 8 cm and bottom measurements at 16 cm below the surface of the water.

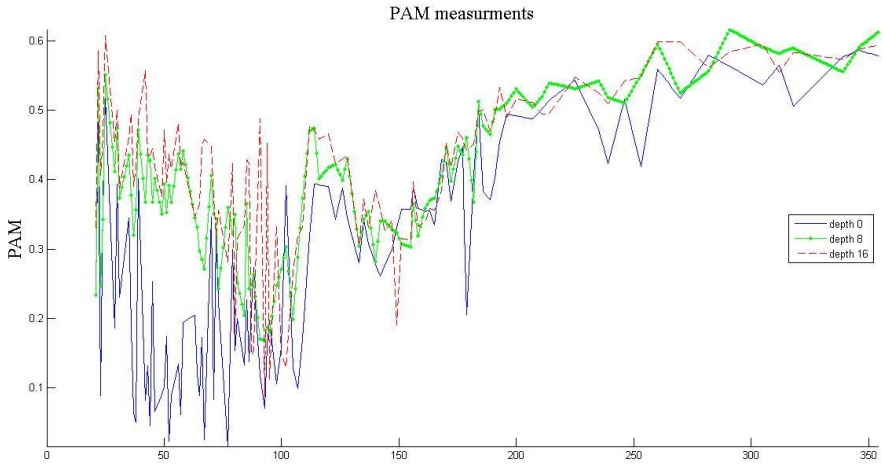

Figure 12- PAM measurements

J. DIC

Dissolved inorganic carbon (DIC), the sum of gaseous CO2, bicarbonate and carbonate dissolved in the water, was the major carbon reservoir in the raceway ponds. Total inorganic carbon is measured by the acidification of the sample which drives the equilibrium to CO2. 1mL of sample were collected from each raceway in 15 mL serum bottles and immediately sealed with rubber caps and aluminum seals. Each bottle was acidified with 0.25 mL of 85% phosphoric acid. When samples containing DIC were subjected to an acidic environment, all DIC is forced into the gas phase as

CO2. The CO2 is then analyzed by gas chromatography (with a Thermal Conductivity Detector). Samples were run weekly using a Shimadzu Gas Chromatograph GC-14A. DIC is measured in mM. It should be noted that these measurements were only taken for the first 126 days.

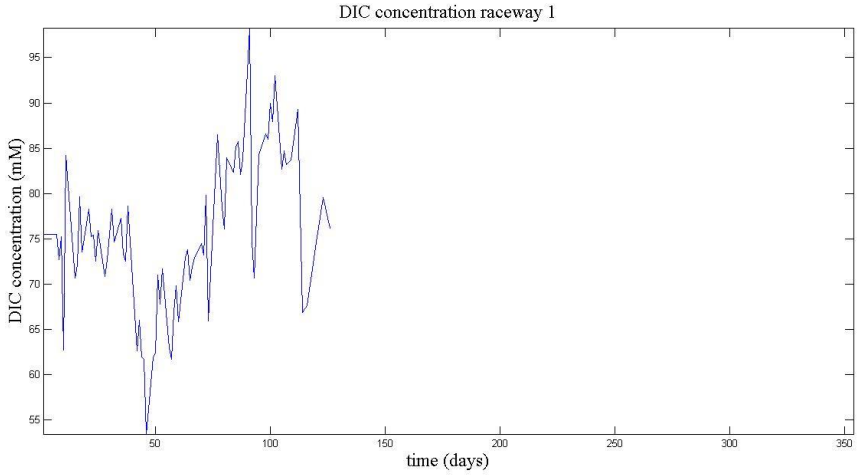

Figure 13 - DIC measurements (first 126 days)

Data Format

Data are organized in a matlab file as a 1x3 struct array with fields:

- irradiance
- temperature
- pH
- oxygen
- DIC
- nitrate
- phosphate
- chlorophyll_a
- density
- salinity
- PAM0
- PAM8
- PAM16
- comments

Each struct array is dedicated to a particular raceway

Each of the variables (irradiance, pH, etc.) has entries data and (normalized) time, e.g., algae(1).pH.data: [48x1 double]
time_num: [48x1 double]

where

"data" contain the raw measurements
"time_num" is the time baselined to the beginning of the experiments on 2/2/2010 (in units of days).

Acknowledgments

The information in this readme file has been compiled from a number of intern reports. Special thanks go to Jasmine McDaniel [8], Nick Myers [9], and Jamie de la Hoz [10] for their help in running the experiments and compiling results.

References

[1] Richmond A. *Handbook of Microalgal Mass Culture*.1st ed., CRC Press, Boca Raton, FL., 2004, p.51
[2] Belov A. P. and Giles J. D."Dynamical model buoyant cyanobacteria". *Hydrobiology* Vol. 349. No. 1-3.1997.pp. 87-97
[3] Chaumont, D. Biotechnology of algal biomass production: a review of systems for outdoor mass culture. *J. Appl. Phycol.*, Vol.5,1993.pp. 593-604.
[4] Oswald, W. J. "Current status of algae from wastes". *Chem. Eng. Symp.*Vol.65.1969, 87-92.
[5] Benemann, J. R. "The future of microalgal biotechnology". *Algal and cyanobacterial biotechnology;* Cresswell, R. C., Res., T.A. V., Shah, N., Eds.; Longman Scientific and Technical Press: Harlow, U.K., 1989; pp 317-337.
[6] Anderson, R. A., *Algal Culturing Techniques*, 1st ed., Elsevier Academic Press, Burlington, MA, 2005, pp. 25-26.

[7] Herrero A. and Flores E. *The Cyanobacteria: Molecular Biology, Genomics and Evolution* .1st ed. Caister Academic Press.2008. pg. 21-22.
[8] J. McDaniel, B. Bebout, A. Detweiler, E. Fleming, and K. Goebel, Growing Spirulina platensis as a model to predict population crashes in open raceway ponds, NASA USRP – Internship Final Report, 2010
[9] N. Myers, Algae Experiments, NASA Ames Research Center Internship Final Report, 2010.
[10] J. de la Hoz, Health assessment and management of algal biomass, Mémoire de fin d'études septembre 2013, NASA Internship Final Report, 2013