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Department of Biology
3rd Scientific Retreat

Research Unit: Plant Biology

An experimental setup to study by remote sensing analyses cyanobacteria growth and photosynthetic performances under non-terrestrial simulated environments

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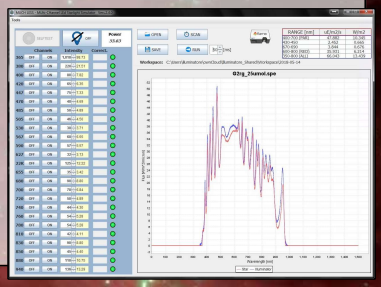
The Project Aim

Many Terrestrial-like exoplanets have been found orbiting the Habitable Zone (HZ) of M-type stars (also known as Red Dwarfs). These celestial bodies are 10 times less luminous than the Sun and have a deeply different light spectra, with a very poor emission in the visible and a major component in the far-red and infrared. The objective of the project is that of simulating the light spectrum reaching the exoplanets orbiting M-type stars, to understand if cyanobacteria could maintain under that condition their oxygenic photosynthetic activity and furthermore impact on primeval atmospheres lacking oxygen.
To this end, the Department of Biology of Padova, INAF and IFN developed the Star Light Simulator (SLS), the Atmosphere Simulator Chamber (ASC), and a newly designed experimental setup to evaluate cyanobacteria growth and photosynthetic performances from remote. For the first time the Normalized Difference Vegetation Index (NDVI), used by satellites to assess vegetation growth on Earth from the space, is applied to a lab-scale system. The system is reliable and matches other physiological measurements taken at the beginning and at the end of the experiments, such as optical density (OD) of the culture, pigment content, dry weight.

Star Light Simulator (SLS)

- 25 channels, 365-940 nm
- 273 air cooled diodes
- Each channel tunable through MUCH_LESS custom-made software
- Reproduces the radiation of F/G/K/M spectral type stars

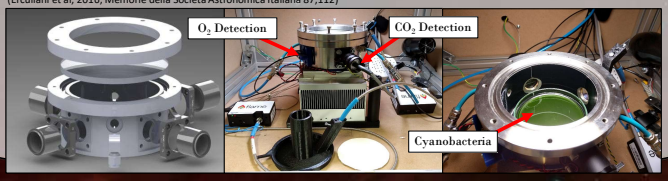
(Salasnich et al, 2018; DOI: 10.1117/12.2311436)



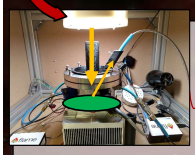
Atmosphere Simulator Chamber (ASC)

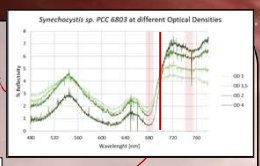
- 0,5 L sealed stainless steel growth chamber
- Borofloat window (SLS approved!)
- Temperature, Pressure and Atmospheric Composition tunable
- % CO_2 measured through *Tunable Diode Laser Absorption Spectroscopy* (TDLAS)
- % O_2 measured through *Oxo-fluorescence*
- Reflectivity measured through Spectrometry

(Erculiani et al, 2016; Memorie della Società Astronomica Italiana 87;112)



Reflectivity detection System





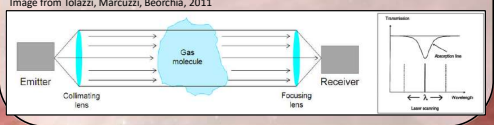
$$NDVI = \frac{Ref(745\ to\ 755nm) - Ref(675\ to\ 685nm)}{Ref(745\ to\ 755nm) + Ref(675\ to\ 685nm)}$$

- Probe collects reflected light from growing cyanobacteria culture
- Spectrometer acquires Photon Counts
- Data is elaborated into Reflection Spectra
- A Normalized Difference Vegetation Index (NDVI) is measured

CO_2 detection System

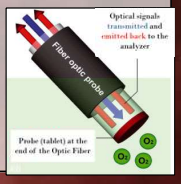
- *Tunable Diode Laser Absorption Spectroscopy (TDLAS)* Technique
- Selected a Single absorption line for CO_2 in the NIR
- Diode laser scans its wavelenght across the absorption line
- CO_2 molecules absorb emitted light at the specific absorption line
- Amount of % CO_2 in the measurment path is calculated

Image from Tolazzi, Marcuzzi, Beorchia, 2011



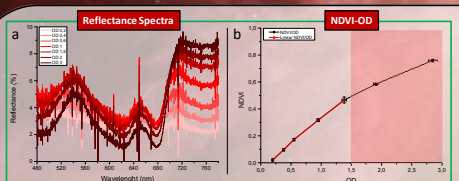
O_2 detection System

- *NomaSense O_2 P300* (Vinventions), used in wine quality assessment
- Based on *Oxo-Fluorescence* Technique
- Blue light transmitted through fiber to a tablet inside the ASC
- Tablet reacts according to dissolved O_2 in the ASC
- Red light emitted back
- Amount of % O_2 is measured
- Detects up to 15 µg/L of O_2



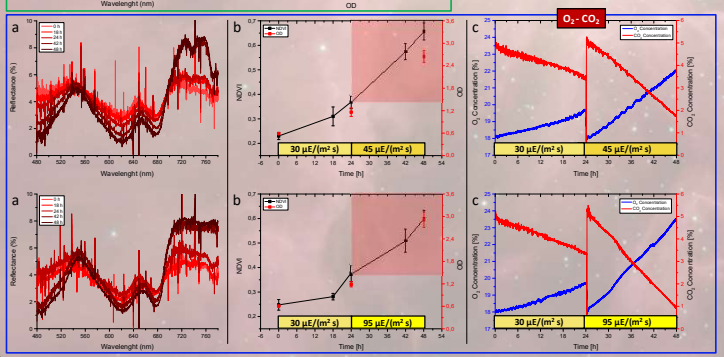
https://www.vinventions.com/it/wine-quality-solutions

Calibration and Validation Experiments with *Synechocystis sp. PCC 6803**



Green Box: Calibration of NDVI measure with *sp. PCC 6803* cultures at different OD

- Reflection spectrum of the culture (a) changes according to different optical densities
- The relation between NDVI values and Optical Density (b) for *sp. PCC 6803* is linear (red line) up to OD = 1,5 OD, beyond that value the linear relationship is lost (red area), so NDVI isn't anymore a good proxy to follow the growth of this organism. Similar linear relationships of NDVI values and Dry weight and/or Chlorophyll a content were also found (Data not shown).



Blue Box: Validation Experiments with sp. *PCC 6803* inside the ASC*

- Reflection spectrum of the culture (a) changes at different time points
- NDVI values over time (b) are in agreement with OD measures below the OD treshold
- Growth of cyanobacteria further confirmed by:
  - O_2/CO_2 charts (c)
  - pigment content and dry weight data at t_0 and t_f (data not shown)
- O_2 evolution and CO_2 consumption changes according to the given light, at a rate that is dependent on the light intensity

***Operational Parameters used in the ASC:**
***T** = 30 °C; **P** = 1 atm;*
***Light Spectrum** = Simulated G2 (Solar) Light;*
***Atmospheric Composition** = 75 %N_2, 20 %O_2, 5 %CO_2*

Conclusions

Our experimental setup is able to give real-time information about the physiological responses of cyanobacteria by remote sensing measurments.

This setup is currently being used to follow the growth of *Synechocystis sp. PCC 6803* and another target species (*C. fritschii sp. PCC 6912*) under both a G2 (Solar) light spectrum and a M-type light spectrum, along with modified atmospheric compositions lacking O_2 and with increasing CO_2 concentrations (in N_2), or with pure CO_2.