Interpreting EChO's future data: biological laboratory estimates under M star's planetary surface conditions

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ABSTRACT

The EChO Exoplanet Atmosphere Characterization mission will have in the midst of its main targets, planets that orbit M stars in their or very close to their habitable zone. In this framework at the Astronomical Observatory of Padova (INAF) we are going to perform experiments that will give us an idea about the possible modification of the atmosphere by photosynthetic biota present on the planet surface. In the framework of the project "Atmosphere In a Test Tube", planetary environmental conditions are being performed. The bacteria that are being studied are *Acaryochloris marina*, *Chroococcidiopsis* sp., *Cyanidium Caldarium* and *Halomicronema hongdechloris* and tests are being performed with LISA ambient simulator in the laboratory of the Padova Astronomical Observatory.

Keywords: EChO, Exoplanet, Atmosphere Characterization, Climate simulation, photosynthetic bacteria

1. INTRODUCTION

EChO, the Exoplanet Characterization Observatory, is a mission concept specifically geared for investigation of exoplanetary atmospheres. EChO will probe the atmospheres of extrasolar planets combining three techniques, making use of planet transits, secondary eclipses, and planet phase-variations, which will also be used for non-transiting planets. In the first case it will perform measurement of the upper part of the planetary atmosphere by means of the transmission spectroscopy technique.
Transmission spectroscopy allows to infer the main opacity sources present in the high atmosphere of the planet (Brown, 2001[1], Tinetti et al. 2007[2]) along the line of sight. It possible only when the planet transits its host star.
Emission spectroscopy (Charbonneau et al. 2005[3]) gives evidence on the thermal structure of the planetary atmosphere and the emission/reflection properties of the planetary surface. It is made observing the daily hemisphere of the planet and exploiting its occultation during the secondary transit.
Recently, a lot of transit surveys have been dedicated to search for Earth size planets planets around M stars and this work is aimed to find Super Earths in the habitable zone.
In this framework it is interesting to search for biosignatures in the atmosphere of these new worlds and to understand how the irradiation quality of a M star modifies (if it does it) the oxygen production of photosynthetic bacteria. Furthermore, for future search, to study the impact of this on the different biosignatures like O_3 and red edge in the spectrum of the atmosphere of super earths orbiting inside the Habitable Zone of an M star.
The "Atmosphere in a Test Tube" (AMT ITT) project is going to do this using an environmental simulator tunable in temperature and gas mixture pressure.
It proposes to utilize this environmental simulator in order to create a database of spectra of warm exoplanet atmospheres simulated in laboratory with a full set of thermodynamic and chemical condition to compare with the future space missions like EChO.
In the following of the paper a description of the experiment and of its aim is given.

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2. SCIENTIFIC REQUIREMENTS

In order to simulate the super Earth atmosphere in the laboratory it is necessary to define some guide parameters, first of all, the value of temperature, and to understand the processes that build the chemical composition of its atmosphere.
As said before, the focus of our research is to simulate super Earths orbiting in the habitable zone of its host star.
This is a crucial statement, because it defines the temperature range, that has to be in the a precise interval.
This interval is dependent on the greenhouse effect, that is effective in maintaining the superficial temperature of the planet as high as it can maintain the water in liquid state.
The distance at which the planet have liquid water depends by the host star, the orbital distance of the planet and the planet itself (Kasting et al., 1993[4] and Selsis et al., 2007[5]).
For a super Earth orbiting an M star using both the "Venus" and "Mars" criterion[5] this range is between 0.47 and 0.88 A.U. that corresponds to 216 K, that is the first condensation of CO_2, and 373 K, the water loss limit.
It is important to remember that the superficial temperature of a planet depends by the pressure of the atmosphere too.
The pressure and the gaseous mixture can regulate the air flows[6,7,8] and shift the habitable zone forward and inward the Habitable Zone (HZ) limits.
For the first part of the experiment, in order to avoid extra-expensive laboratory infrastructure and simplify the work, we'll consider super Earths well inside the HZ of the host star with temperatures between 273 K and 288 K, that are the current temperature values on Earth.

2.1 Biomarkers and photosynthetic pigments

Photosynthetic bacteria are the special guests of this experiment and the key to understand how a hot, inhospitable planet can became a safe green and life friendly planet.
In fact it is well known that photosynthetic organisms can produce gases, like O_2 (or O_3 from its photolysis), and nitric oxides like N_2O_2. Other molecules, like NO_X, CH_3Cl or COS are formed from the breakdown of organic matter. All these molecules can have modified exoplanet's atmospheres in time and can be detected from Earth.
Some of them are called biomarkers because they can outline the presence of biotic organisms on other planets.
But as not all that glitters is gold, it is important to remember that biomarker measurements are affected by the false positive problem. In fact, O_2 can be produced even a-biotically through photolysis and the effects of carbon burial and hydrogen escape. Though, its simultaneous presence with other reduced gases as CH_4 can be explained only with biotic processes that maintain chemical disequilibrium (Kiang et al., 2007b[9]). Detectability of photosynthetic processes depends on biotic productivity too, which depends on several factors, like availability of resources like water, light, minerals, electron donors and nutrients. During the photosynthetic process, light impacts on photo-receptive organisms (antennae) that split water molecules and produce proton gradients and energy useful for the cells. As O_2, O_3 and CH_4 are the main biomarkers, as well as NH_3 and N_2O, it is crucial to understand how organisms can operate photosynthesis on M star planets and if the metabolic processes can be influenced by different types of radiation. In fact, a detectable missing concentration of O_2 and/or O_3 in combination with reduced gases like CH_4 is a strong signature of biologic activity, as explained in Lammer et al., 2009[10]. That's why in this experiment we will use photosynthetic bacteria. It is important to remember that not all bacteria respond equally to irradiation, but all of them have a typical response to light spectrum. The theoretical unicellular lower light limit useful to operate photosynthesis, estimated by Raven (1984)[11] is 0.1 μmol of photons/m^2/s (6 x 10^{16} photons/m^2/s). For the upper limit of photon flux density, Wolstencroft and Raven (2002)[12] found that the theoretical tolerance for land plants against photo damage is 6–9 mmol of photons/m^2/s (3.6–5.4 x 10^{21} photons/m^2/s) over the standard Photosynthetic Active Region of the spectrum (PAR, the part of the radiation spectrum between which organisms can operate photosynthesis), well above Earth's typical flux of 2 mmol photons/m^2/s (1.2x10^{21} photons/m^2/s). For Earth-like planets in general, Wolstencroft and Raven conjectured a theoretical upper limit for land organisms to be 10 mmol of photons/m^2/s (6x10^{21} photons/m^2/s). For example, aquatic organisms and cryptoendolithic organisms are shielded under water and under rocks, so they could exist for even higher surface photon flux densities (Kiang et al., 2007a)[13]. First of all it is useful to make a brief description of photopigments. All photosynthetic organisms have photopigments that can harvest light:
These are chlorophylls like Chl a that occurs in the core antenna, or other Chls, Chl b, c and d, that provide light harvesting roles. Chl d, recently discovered in cyanobacteria (Miyashita et al., (1996)[14]; Miller et al., (2005)[15], Mielke et al., (2011)[16]), may replace Chl a in some cyanobacteria that live in environments with little visible light (Chen et al., (2005)[17]; Larkum and Kühl, (2005)[18]).

Chl d has its major peak absorbance in the NIR at 720 nm (Manning and Strain, (1943)[19]; Larkum and Kühl, (2005)[18]), and thus oxygenic photosynthesis is being performed in the NIR (Kiang et al, 2007a) [13]. Recently Chl f has been discovered which is able to capture light energy in the infrared spectrum, with an absorption peak at 706 nm.
In non oxygenic bacteria, BChls play a primary role in electron donor and their peak can extend through the NIR part of the spectrum. Other photopigments are carotenoids and phycobilines. The first work in the blue and green part of the spectrum and helps organisms to protect themselves against photooxiadtive stresses, high temperatures and the toxic presence of O_2. Phycobilines, can be found in cyanobacteria and red algae, and work in the green and yellow spectral regions. Other living organisms, such as green bacteria, purple bacteria and heliobacteria, can exploit solar light in slightly extended spectral regions or in ecological niches, such as the near-infrared. For example, purple bacteria have absorbance peak in the 1.013-1.025 μm range using BChl b like *Blastochloris viridi* or *Rhodopseudomonas viridis* (that absorbs at 0.96 μm) and other bacteriochlorophylls in the range 0.7-0.9 μm (Scheer, 2003)[20]. They don't use water as H donor, and then don't release oxygen as byproduct. PAR on M star planets can be lower than the average terrestrial value ever by an order of magnitude (Heath et al., 1999)[21]. Nevertheless this could not represent a problem because several marine organisms on Earth evolved to use only $5x10^{-4}$ times the average flux received at the Earth's surface, like sulfur bacteria that embed a large antenna complex, the chlorosome, that permit to use only small fractions of light intensities (McKay, 2000)[22]. In these regions radiation is dominated by red or IR radiation.

2.2 Photosynthetic bacteria

For our purpose we would need a bacterium that is resistant to harsh conditions (maybe an extremophile one) and that is capable to photosynthetize in the NIR. The choice has been fallen on some types of oxygenic bacteria.

Cyanidium Caldarium is a microscopic red algae found in acidic hot streams, and moist acidic soils all over the world. Algaes like *Cyanidium caldarium* use photosynthesis to acquire their nutrients but, unlike the typical green algae, contain the photosynthetic pigment, phycoerythrin, in addition to chlorophyll.

Chroococcidiopsis spp. is a desiccation-tolerant chasmoendolithic coccoidal cyanobacterium and it is one of the most primitive cyanobacteria known with a doubling time (t_D) in normal growth conditions is 3-4 days. It is good for its ability to survive harsh environmental conditions, including both high and low temperatures, ionizing radiation, and high salinity but doesn't have an extended NIR sensitivity. In figure 1 is shown a picture of it (courtesy of Antje Donner, (2013)[23]). According to Billi et al., (1996)[24], O_2 production (evolution–uptake) has been recorded recorded to be around 40 fmol/cell/h in normal growth condition (20 μmol of photons m^2 s^{-1}). Taking as approximation a typical bacterial cell mass around 1pg this would lead to a production of about 40 mmol/g/h. Its theoretical light limit for minimum photosynthetic production is $0.1x10^{-6}$ mol of photons m m^2 s^{-1}.

Acharyochloris marina is a symbiotic species of the phylum Cyanobacteria and is another good choice because it produces Chlorophyll d, allowing it to utilize far-red light, at 710 nm wavelength. In Behrendtet al, (2012)[25] it has been found that t_D is 1.82 days between 400 and 700 nm and 1.09 at 720 nm. For this reason, O_2 production is $1.272x10^{-6}$ mol O_2/mg Chld/h in the NIR and $1.128x10^{-6}$ mol O_2/mg Chld/h in the VIR range. In figure 2 is shown a picture of it.

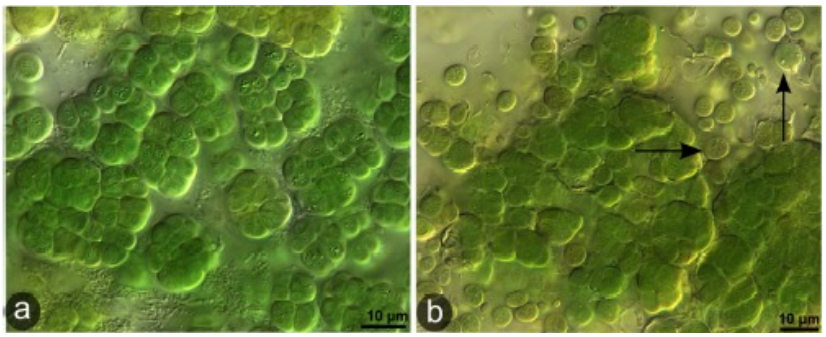

Figure 1. Light micrographs of *Chroococcidiopsis* grown on BG 11 or BG 11 liquid medium: cells and aggregates at different stages of development (C. sp. BB 81.1) (a) and vegetative undivided cells (arrows, C. sp. 96.1) (b) (courtesy of Antje Donner, (2013)[23]).

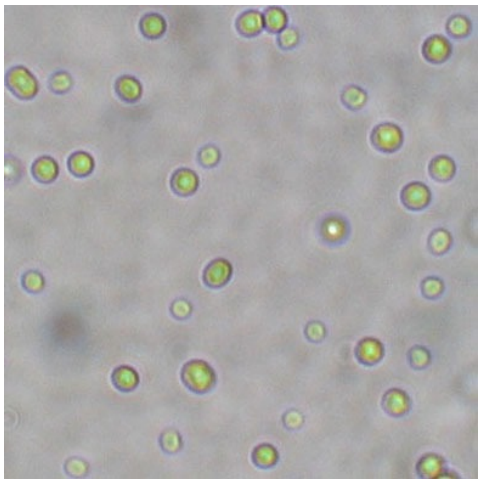

Figure 2. Photomicrograph of *Acaryochloris marina*. Credit: Phototrophic Prokaryotes Sequencing Project, via NCBI

On the other hand we are studying *Halomicronema hongdechloris* too, a filamentous cyanobacterium containing chlorophyll f. A picture of it is show in figure 3.

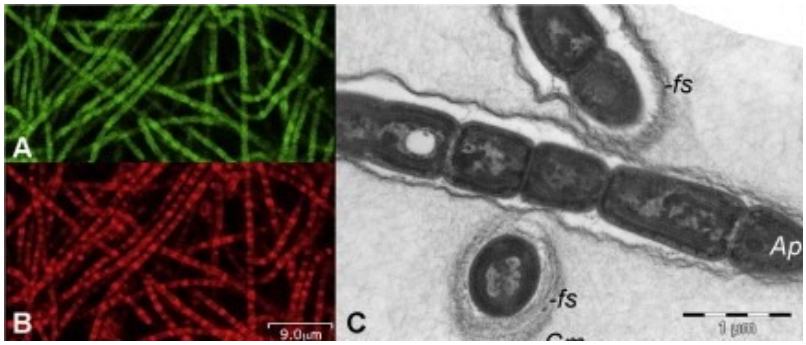

Figure 3. Confocal light microscopic and transmission electron microscopic photography. A and B, confocal light microscopic images of *Halomicronema hongdechloris* cells by $\lambda_{ex} = 488$ nm, and $\lambda_{em} < 565$ nm LP filter (A) and $\lambda_{em} < 660$ nm LP filter (B) (form Chen et al., (2012))[26]

For a good calculation of the metabolic evolution in time there would need many parameters. In fact, studies show that bacteria O_2 productivity is directly dependent on bacteria growth and pigment concentration as well as the light irradiation. Bacterial growth can be fitted with a Gompertz curve with three freedom degrees.

3. THE EXPERIMENT

The instrument that will be used to carry out the experiment is LISA-SAM. LISA-SAM has originally been created by the Astronomy Department of University of Padua to study how living bacteria, mosses and lichens could survive in a Martian atmosphere. The main structure is a steel cylinder inside which are located six cells (inside which biological samples can be placed) with a 250 cm^3 capacity (0.25 l) and topped by a Suprasil glass window transparent from UV to NIR (see figure 4).

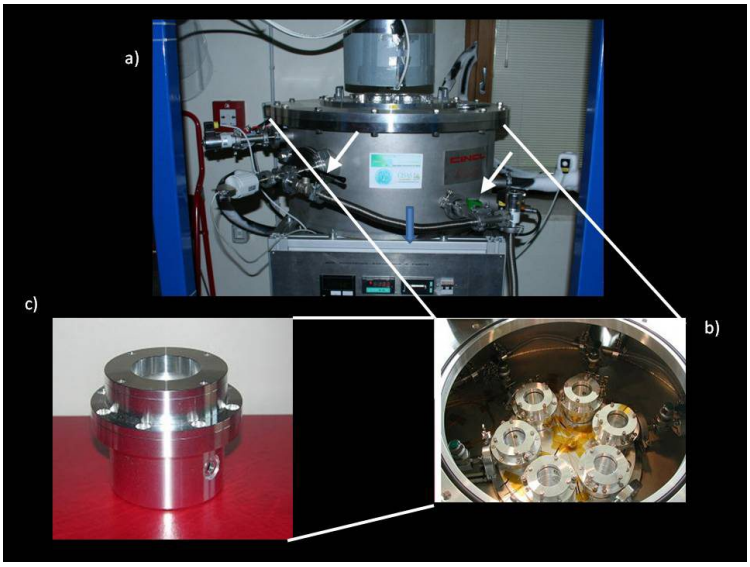

Figure 4. In the picture can be seen the instrumental complex in toto (a) and the particular of one of the six cells located inside it (b, c). The LISA SAM has been built by the Astronomy Department of University of Padova

Cells are connected with the outer part by pipes at the end of which are implemented mechanical filters to let gas mixture to course and at the same time avoid biological material to go through the pipes inside the cryostatic chamber located at the base of LISA-SAM. Until now, the cooling process have been made by contact, with an aluminium plate located on the liquid nitrogen reserve (Ranger Air Liquide), but as biological samples should be kept at a mean temperature of 293 K, there is no need of liquid nitrogen to reach this temperature and this goal could be easily achieved with a Peltier cell kept under the cells. This way the temperature can be raised or lowered. For practical and energetic reasons we are trying to understand how to customize MINI-LISA, an instrument similar to LISA but smaller and with an unique cell for lodging biological samples, to perform a single sample experiment. In figure 5 is shown a picture of the internal part of MINI-LISA. As can be seen, a standard commercial Dewar (from Oxford Instruments) modified for our applications represents the main body.

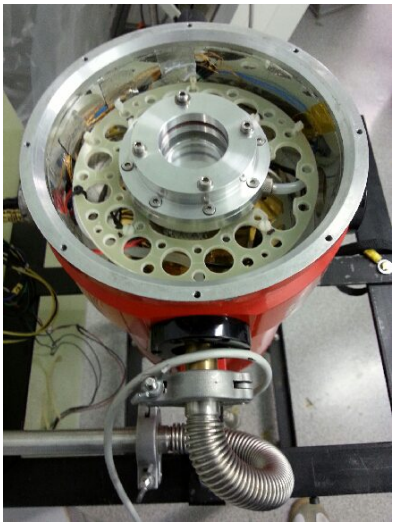

Figure 5. Internal part of MINI-LISA.

As shown in Figure 5 the reaction cell is isolated from the rest of the Dewar. This allow to evacuate the space between the cell and the Dewar walls by the use of a vacuum turbo-pump. Respect to LISA-SAM, MINI-LISA has only one reaction cell and so, to allow the presence of a comparison sample in the same reaction cell, can be used a special combination of aluminum dishes stacked up one upon the other and inserted in the bottom of the reaction cell, similar to the classical Petri dishes for biological samples (Galletta et al., 2010)[27]. These plates will have to be substituted because aluminium can damage photosynthetic bacteria[28]. This is one of the reasons why, as will be described in chapter 4 cells will be make of stainless steel. The first preparatory and preliminary tests will need to test the hardware, in particular to understand how the cells keep pressure and void air tightness. This gave us an estimate of the limits in sensibility our system can reach. Done this, will begin the main part of the experiment, that consists of three steps. In the first step, called the fiduciary experiment, measurements of photosynthetic bacteria products will be taken in terrestrial conditions. It's obvious that the choice of these bacteria will be done depending on their productivity rate and metabolic processes as previously said. The other choice parameter is their resistance to extreme environments. This part of the experiment will be surely done in a biological laboratory because of the need of a controlled environment chamber to lodge bacteria inside the cells. In the fiduciary experiment, cells will be filled with a gaseous mix that will reproduce Earth's atmospheric composition. During the first step, the pressure will be kept constant at 1.013 bar (Earth's mean pressure at sea level) and the temperature will be around 298 K, a temperature suitable for oxygenic photosynthetic bacteria. The irradiation will reproduce the solar irradiation spectrum. To be still defined is the choice of one unique lamp of 100 W or 6 lamps of 3 W dedicated for each cell. This will depend on the lamp costs and lamp power need. In order to avoid frosting damages, void will be kept inside the chamber around the cells. Before and during sample irradiation will be taken measurements of gas composition, expecting a peak of gas concentration variations at the end of bacteria metabolic and photosynthetic process. The measurements of gas concentrations inside the cells will be done with a Tunable Diode Laser Absorption Spectroscopy setup (TDLAS) and is based on absorption energy following Beer's law. This measurement method provides a Vertical Cavity Surface Emitting laser (VCSEL) source shot through the cell environment and to a photodiode diode laser tuned to a particular narrow emission band. The source wave number is selected in order to match a single absorption line on a molecule of interest and the laser emission is scanned several times across the whole spectral width of the absorption feature. Usually, the line width of the laser emission is much smaller than the molecular absorption line width allowing the instrument to be selective among components of a gas mixture and have no interferences from other gases, especially at low pressure (the absorption lines are narrower). The sensitivity of the analyzer is dependent on the absorption strength of the line chosen and on the absorption path length[29]. Many gases of biological interest can be sensed in this way, for example HF (detection limit 0.2 ppm.m), H_2S (detection limit 20.0 ppm.m), NH_3 (detection limit 5.0 ppm.m), H_2O (detection limit 1.0 ppm.m), CH_4 (detection limit 1.0 ppm.m), HCl (detection limit 0.15 ppm.m), HCN (detection limit 1.0 ppm.m), CO (detection limit 40.0 ppm.m), CO_2 (detection limit 40.0 ppm.m), NO (detection limit 30.0 ppm.m), NO_7 (detection limit 0.2 ppm.m), O_2 (detection limit 50.0 ppm.m). A Wavelength Modulation Spectroscopy scheme will be used to improve detection of weak absorptions from the low concentrations obtained. The measurement setup will be based on a PC with a DAQ card for synchronous modulation and demodulation of the WMS waveforms as well as for fitting the absorption signals. In figure 6 is shown a scheme of its working.

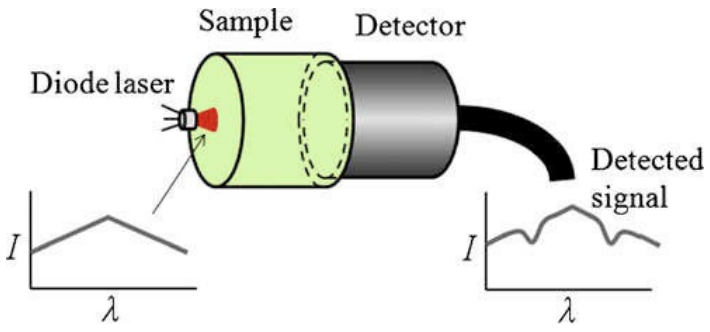

Figure 6. Working scheme of the gas detector.

The second step of the experiment will consist to change the irradiation source to simulate an M star's one on the surface of the planet. This irradiation lamp still need to be realized. M star spectra have strong absorbance lines, so it is difficult to reproduce exactly it as they are not good black body emitters. Finally, in the third step we will change even the gas mixture inside the cells, keeping the irradiation source as in the previous step. The gas composition will come by theoretical simulation of super earths atmospheres.

4. STATE OF THE ART

The experiment is evolving on more than one front, the more challenging of which are the test and customization of existing hardware (the step zero), the development of the irradiation source and the choice of the samples.

4.1 Test of the cells

All the cells have been tested with a Helium leak detector and the scale of Helium leak is between $7x10^{-7}$ and 10^{-9} mbar. Then, all the cells have then been tested in vacuum with a diaphragm 4 m^3/h pump and an ALCATEL turbo molecular 250 m^3/h pump, covering the external bolts with a Teflon binding when necessary. All cells show cells show the same void trend except cell 1 that shows a little different trend because increasing the time increases even the error (the two points at 80 and 90 h). Though, as the time we will use to carry out the measurements with the biological samples won't exceed two days, these data are not relevant. The pressure measurements are shown in figure 7. The main parameters to keep under control are the time constant τ, calculated as

$$\tau = \frac{t}{\ln[\frac{k}{P_o - P(t)}]} \quad (1)$$

where P(t) is the pressure at time t, P_0 is the pressure of the air, k is the difference between P_0 and P(t=0), the first measure kept close to the vacuum. The loss rate Q_A is calculated as

$$Q_A = \frac{P_0}{tV} \quad (2)$$

where V is the cell volume (250 cm^3=0.25 l). In particular has been found that cell1 has a time constant τ =1505.42 h and a Q_A=0.166 mbar l h^{-1}, for cell 2 τ =2030.59 h and Q_A=0.123 mbar l h^{-1}, for cell 3 has τ =1934.72 and Q_A=0.129 mbar l x h^{-1}, for cell 4 the time constant τ is 2242.21 h and Q_A=0.112 mbar l h^{-1} and cell 5 have the time constant τ =2114.16 h and Q_A=0.118 mbar l h^{-1}. More studies, even in over pressure, are required to understand if these lacks are as low for the biological gaseous productions to be detectables. The Suprasil glass characterization is an important part of the experiment to understand which component of the spectrum can be transmitted from it and can impact onto the biological samples. The measurements have been carried out by means of a spectrometer and shown in figure 8. The mean value of transmittance for the glass is 0.88±0.082, according with the literature. Lodging cells inherited from the previous experiments are made of aluminium. This could be a problem, as previously said, because this material can damage bacterial vitality and influence their photosynthetic processes [29]. In order to reach best results we projected a new set of cells with a couple of flanged hoses useful for the gas measurements, and wholly made of stainless steel, as shown in figure 9. The cells are now being realized.

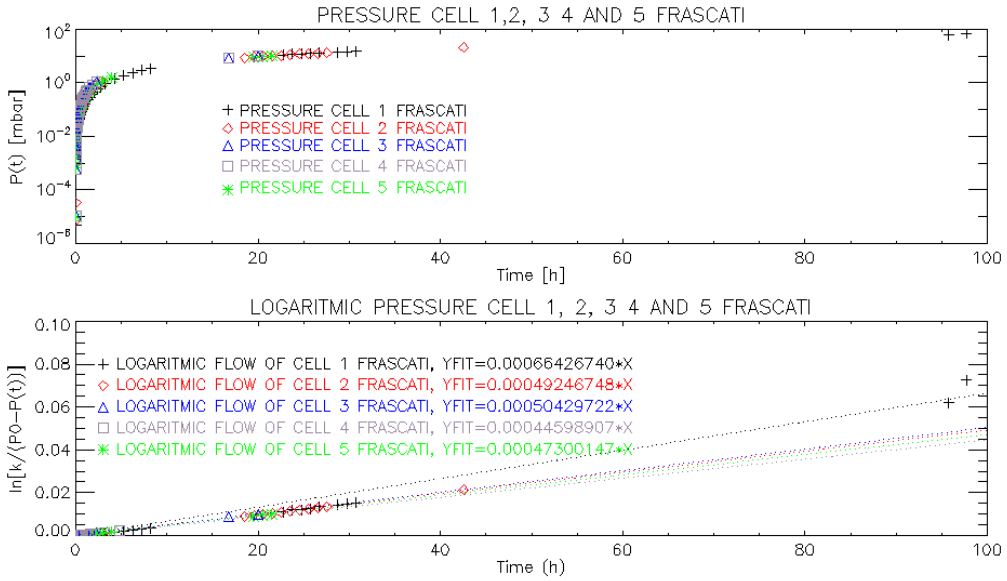

Figure 7. Cumulative vacuum pressure measurements plot of all cells

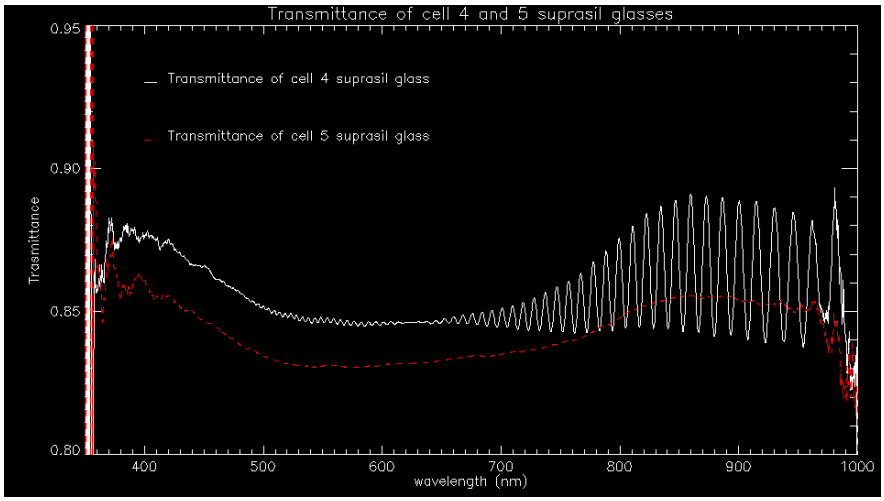

Figure 8. Cumulative Transmittance of cell 4 and 5 Suprasil glasses

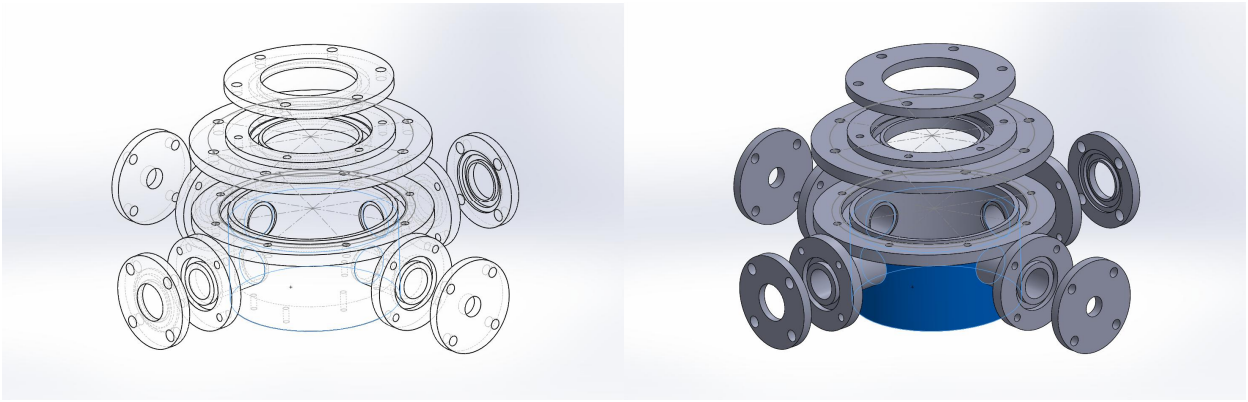

Figure 9. New project of the cell. Drawings realized with SolidWorks.

4.2 Illumination system

The illumination of the samples the key to carry out the experiment. As already said, the biological samples will have to be irradiated first with a solar light radiation and then with an M star radiation. Our goal is to build a stellar light simulator capable to recreate the radiation spectrum of an F, G, K and M type star. To do this is essential to analyze which kind of radiation sources are available on the market and to compare them in order to choose the most suitable ones for our research. Done this, is crucial to fit the radiation spectrum provided by the source with the blackbody radiation curve (processed or not by the planetary atmosphere) of the selected star, so as to match the first with the second. It is prominent to underline that, as we aim to irradiate photosynthetic bacterial samples, not all the whole spectrum will be useful, but only the one included in the wavelength interval enclosed between the inner and the outer limits of photosynthetic pigment absorption range. In figure 10 (a,b) are shown the different black body radiation spectra at different temperatures, corresponding to F, G, K and M stars with superimposed the absorption spectra of some of the principal photosynthetic pigments used by photosynthetic bacteria. Up to now have been compared different light sources as lamps and LEDs. Lamps as Tungsten-Halogen, Mercury, Xenon and Metal-Halide are the most common radiation sources on the marketplace. They are broadband devices with an emission spectrum that can cover different wavelengths. The advantage of lamps is their high luminosity and broadbandwidth, but their disadvantage is the stillness of the spectrum and the impossibility to change it in real time. Though, the Tungsten-Halogen and the Xenon lamps are the only capable to emit in the NIR bandwidth (the peak of M type stars). Adopting broad limits we have a lower limit of 280 nm, under which no photosynthetic process is possible, while the higher limit is defined by the ability of the photosynthetic bacteria to absorb the radiation. For the fiduciary experiment, the first step of this research each kind of these lamps could be useful. In particular it would be suitable a Xenon Arc lamp, in particular the ORIEL 6258 300 Watt Ozone Free one used in Galletta et al., 2007[30]. Other measurements should be made to understand the radiant spectrum in definitive conditions even for $\lambda < 500$ nm. Then, to represent the M star's planets we are studying a tunable LED solution. In fact they are cheap, small and more versatile. For our purpose, one of the best feature of LEDs is that it is possible to switch it on or off driving it by a PC. This is a great advantage respect to lamp illumination, that have a fix spectrum, because an array of LEDs could be used to match the desired part of the spectrum lightening them in sequence. In order to build a radiation source than can change the radiation spectrum with time the LED solution is a good idea, but it carries some not negligible problems suck the irradiation cone, the low power and the spectral coverage. For the first problem, an idea of a possible configuration could be fixing them inside a conic device that can converge light on the top of the windows of the cells. Then, as said, each LED has a different irradiation angle that depends on it emissivity and power. Optical fiber could be a good alternative to avoid LED's encumbrance. These devices are very compact and could be easy to build a bundle with different irradiation wavelength and keeping it in a restricted space. The problem with the optical fibers is the radiative power. A solution could be a new fiber technology named Hollow core fibers (NKT photonics). These devices have a design that uses a hollow, air-filled core that removes length limitation and dramatically improves performance by forcing light to travel through channels of air, instead of the glass around it. They also have lower signal loss [31]. In every case, whether we are using fibers or LEDs, is important to uniform the beam in order to have a flat illumination avoiding the cone effect due to each single device.

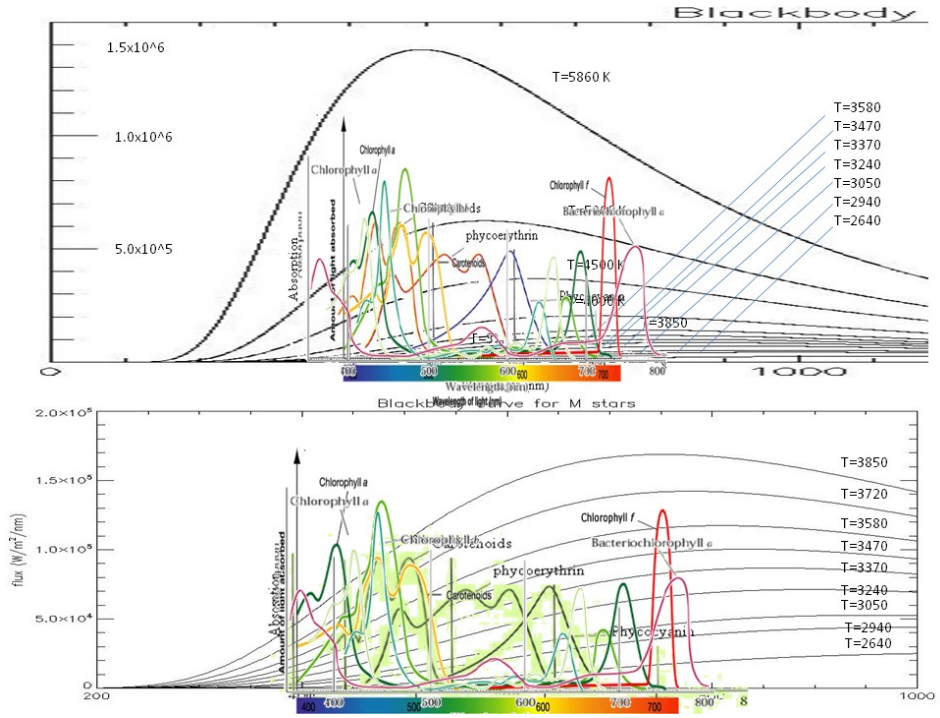

Figure 10. different black body radiation spectra at different temperatures, corresponding to F, G, K and M (a) and only M (b) stars with superimposed the absorption spectra of some of the principal photosynthetic pigments used by photosynthetic bacteria (from http://biochimicadelmetabolismo.wordpress.com/tag/risposta-alla-luce/ and Treccani Online Enciclopedy).

5. CONCLUSIONS

In this work we have described the ongoing project of an experiment focused on the study of biosignatures in the atmosphere of a super Earth orbiting an M star well inside its habitable zone. We have underlined the scientific requirements and choice of the bacteria. We have therefore analyzed the aspects of hardware customizing and the development of a source of irradiation.

In setting up the experiment we are talking with some difficulties at which we are giving answers but with good probabilities the experiment will star in fall with its fiduciary phase.

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