Development of a testbed for flow-through measurements of algal metabolism under altered pressure for bioregenerative life support applications

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The utilization of algae is a widespread concept for bioregenerative life support systems in human spaceflight. Algae have the potential to combine the functions of air revitalization, wastewater treatment, and food production via photosynthesis. The potential benefits of using algae include high reliability, reduced mass, and psychological benefits to the crew. Due to the fast growth rate and ease of culturing, *Chlorella vulgaris* is well documented in terms of optimal growth parameters, such as carbon dioxide or oxygen concentration, growth medium, temperature, as well as light cycle, spectrum, and intensity. However, the feasibility of algal photobioreactors for air revitalization, wastewater treatment, and food production under relevant spaceflight environments is not fully assessed. In particular, algal growth under NASA's proposed exploration atmosphere of 8.2 psia and 34 % oxygen for long-duration human spaceflight missions has not been characterized. Therefore, a flow-through photobioreactor that is capable of maintaining specified growth conditions for *Chlorella vulgaris* and controlling the pressure in the reactor between 56.5 and 101.3 kPa (8.2 and 14.7 psia) was developed and is presented in this paper. The sizing process of the small scale photobioreactor for gaining accurate oxygen and carbon dioxide measurements is described. Additionally, challenges, such as leak rates, measurement resolution, and water temperature alternating the solubility of carbon dioxide and oxygen, are discussed. In conclusion, the adaptations to more typical state-of-the-art, environmentally-open reactor designs, necessary to meet the minimal leak rate requirements for measuring the gas exchanges, are summarized. Preliminary metabolic measurements from the algal photobioreactor testbed are presented. Future characterization studies, using this testbed design, can lead to a better understanding of algal performance and more accurate system analysis for future life support system designs.

Keywords: human spaceflight, bioastronautics, *Chlorella vulgaris*, air revitalization, exploration atmosphere, environmental control and life support system

Nomenclature

BBM	=	Bold's Basic Medium
BLSS	=	Bioregenerative Life Support System
DOX	=	Dissolved Oxygen
ECLSS	=	Environmental Control and Life Support System
ESA	=	European Space Agency
ISS	=	International Space Station
LED	=	Light-Emitting Diode
NASA	=	National Aeronautics and Space Administration
NDIR	=	Nondispersive Infrared
PAR	=	Photosynthetically Active Radiation
TDMS	=	Technical Data Management Streaming

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I. Introduction

IN space habitats, Environmental Control and Life Support Systems (ECLSS) are used to provide habitable conditions for human beings in the hostile external environment. Currently, life support functions are performed using physicochemical systems onboard the International Space Station (ISS), recycling part of the waste water and carbon dioxide to potable water and oxygen[1, 2]. With increasing mission durations and distances from Earth, regenerative life support systems are becoming increasingly important. Through recycling the human waste products into consumables without the need of resupplies, the total up mass for the mission can potentially be reduced. To further close the recycling loop of a spacecraft, bioregenerative life support systems (BLSS) also provide the potential to produce food in-situ[3]. Due to their ability to process human wastes, carbon dioxide, and metabolically produced water to evolve oxygen, filter water, and produce edible biomass in one system, the use of algae is a widespread concept[4]. Potential benefits of such systems include a reduced infrastructure mass due to multifunctionality and high reliability because of biological self-adaptation. Compared to higher plants, algae offer a promising alternative for life support system designs due to their high growth rate as well as their simple processing through isotropic characteristics of the algal solution.

Certain algae, especially the green algae *Chlorella vulgaris* used in this study, have undergone intensive research on the ground to increase growth rates for the biofuel, food, and cosmetic industries. The results obtained from these experiments can be used as a starting point to design algal life support systems. However, in order to implement an algal photobioreactor into closed habitats, further characterizations have to be conducted. This includes studying the effect of radiation, microgravity, and air composition on the algal growth and metabolism. As NASA has proposed an exploration atmosphere of 8.2 psia (56.5 kPa) and 34 % oxygen for future space habitats[5], the effects of reduced pressure on algae is of particular interest[6]. In this project, an algal photobioreactor is developed that is capable of simulating the low pressure and high oxygen fraction environment of the proposed exploration atmosphere for studies on the algal performance in laboratory settings.

II. Materials and methods

A. Concept of operation

Published algal studies found in the literature that were aimed at characterizing the effect of reduced total pressure were interested in the biomass production of algae[7, 8] and did not measure the oxygen evolution or carbon dioxide fixation rate. For life support applications, however, the algal metabolism is of interest, making the typical experimental setups insufficient. Therefore, the newly developed test setup follows a flow-through approach as depicted in Figure 1. A premixed gas mixture with known composition is fed into the variable-pressure photobioreactor that allows gas to exchange between the liquid and gaseous phase. The continuous gas supply also pushes the overflowing gas mixture out of the photobioreactor into the gas analyzer. Analysis of the gas outlet carbon dioxide and oxygen compositions allows for performance calculations. This system has the advantage of exposing the algae to a steady state gas phase that does not get altered over time by the algal metabolism, as it is the case with hermetically sealed, batch-style photobioreactors.

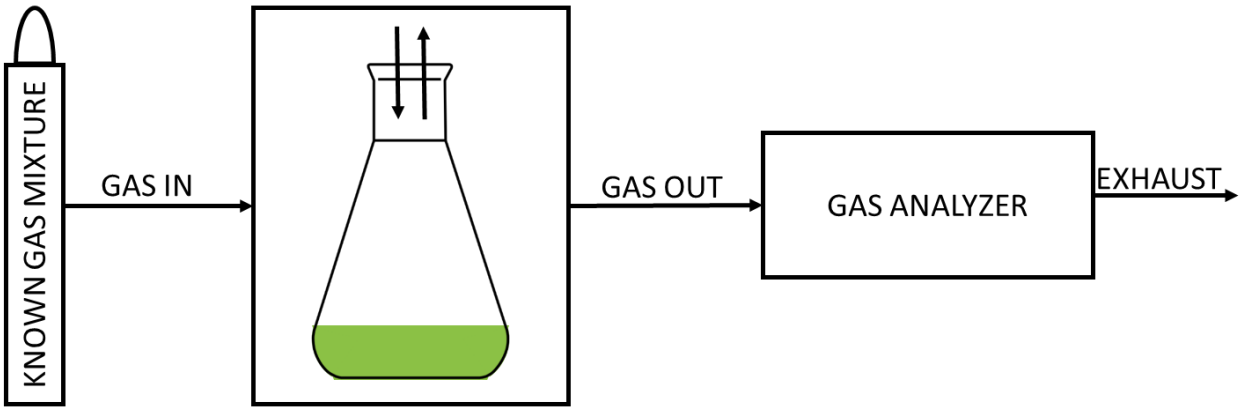

Figure 1: Flow-through experimental test setup schematic

B. Requirements

To understand the influence of the gas exchange on algae, the remaining environmental parameters have to be kept in their optimal ranges to not become a growth limiting factor. Environmental parameters affecting the growth of *Chlorella vulgaris* have been identified in previoius studies [4, 9–11]. The variables with their selected correlating values are shown in Table 1. These values represent precise set points instead of minimum or maximum parameters. As shown in the example of light intensity, algae respond proportionally to low light levels during the light limited phase. At light levels ranging from 200-300 µmol/(m^2·s) they are entering a plateau due to light saturation and are growth limited by other factors. Above 300 µmol/(m^2·s) they enter the light inhibited phase, where growth rate rapidly drops off. A similar behavior is also seen with temperature.

Table 1: Optimum growth conditions for *Chlorella vulgaris*

Variable	Value
Temperature	30 °C[12–14]
Light spectrum	400 – 700 nm[15, 16]
Light intensity (within PAR range)	300 µmol/(m^2·s)[17–20]
Light cycle	12/12 (day/night)[21]
Growth media type	Bolds Basic Medium (BBM)[22]

As the purpose of this experimental setup is to characterize the metabolism of algal cells in a photobioreactor under varying cabin atmosphere conditions, the testbed had to be capable of providing the input parameters in a wide range of compositions and pressures. Oxygen, carbon dioxide and nitrogen concentrations must be provided in ranges preferably between 0 to 100 % at standard sea level conditions. This equals a partial pressure range of 0 – 101.3 kPa for each gas, which is shown in Table 2. Specifically for oxygen, this requirement was limited to 21.3 kPa for flammability concerns. As a special requirement to incorporate NASA's proposed exploration atmosphere, the reactor was designed to be able to operate at total pressures between 56.5 and 101.3 kPa (8.2 and 14.7 psia).

Table 2: Required gas input composition ranges

Variable	Value
Reactor total pressure	56.5 – 101.3 kPa
Carbon dioxide partial pressure	0 – 101.3 kPa
Nitrogen partial pressure	0 – 101.3 kPa
Oxygen partial pressure	0 – 21.3 kPa

C. Design

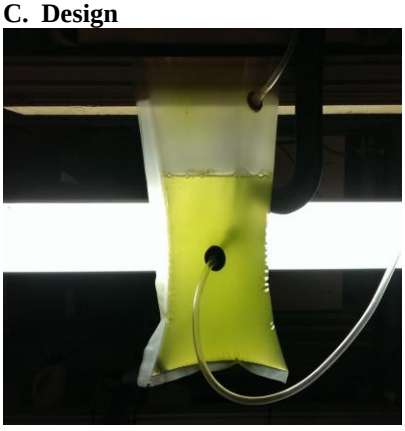

Figure 2: Gas-permeable bag photobioreactor

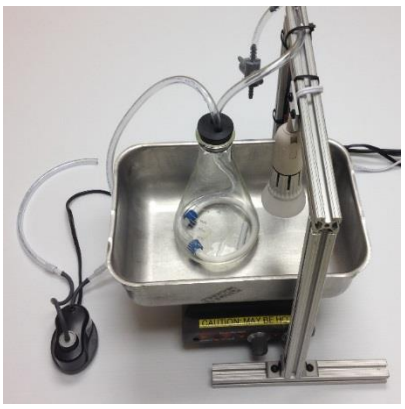

Figure 3: Erlenmeyer flask photobioreactor

Figure 4: Pressure tank photobioreactor

Several different designs were experimentally compared with each other. A gas-permeable bag approach (Figure 2) showed too large diffusion rates for both carbon dioxide and oxygen across the membrane to obtain useful data. Erlenmeyer flasks (Figure 3), which are commonly used to culture algae, do not provide pressure tight interfaces and showed unacceptably high leak rates. In a pressure tank housing (Figure 4), the reactor proved difficult to handle

operationally, as the entire pressure tank had to be opened to troubleshoot sensors, which required stopping the experiment. Additionally, condensation on the electronics within the pressure tank proved to be a challenge. Through the experience gained from these concepts, a benchtop photobioreactor was designed for the purpose of collecting metabolic data under altered pressures as described in the following paragraphs.

1. Mechanical design

To maintain comparability with previous studies[23–26], a tubular photobioreactor design, as shown in Figure 5, was chosen. The central element of the design setup, as shown in Figure 6, is an acrylic reactor in a cylindrical shape which has an internal volume of 2.5 l and is designed to withhold both positive and negative pressures of up to +18 kPa and -28 kPa. This is necessary to perform studies in the absolute pressure range of 56.5 kPa (exploration atmosphere) to 101.3 kPa (standard sea level), while accounting for an atmospheric pressure in Boulder of about 85 kPa due to elevation. The top and bottom of the cylinder are circular disks with integrated O-rings that are kept in place by two internal retaining rings. This allows for fast assembly and disassembly of the reactor to reach the internal surfaces for cleaning purposes. Circling around the photobioreactor, white light emitting diodes (LED) are arranged uniformly over the entire height and deliver a light intensity of 300 µmol/(m^2·s) to the surface of the photobioreactor. White LEDs where used to replicate Earth lighting conditions for the crew. If power is a limiting factor, blue and red LEDs should be used instead to achieve higher energy conversion rates. The components of the test setup between the rotameter and the vacuum pump are within an environmental chamber that controls the temperature to a constant 30 °C. This makes the experiment independent of any day/night cycles of the temperature within the lab. Additionally, the air stream within the environmental chamber cools the LED's and prevents a temperature change of the algal solution due to the light cycle. The bottom plate has an inlet in the center, where a constant air stream of 0.5 l/min is inserted.

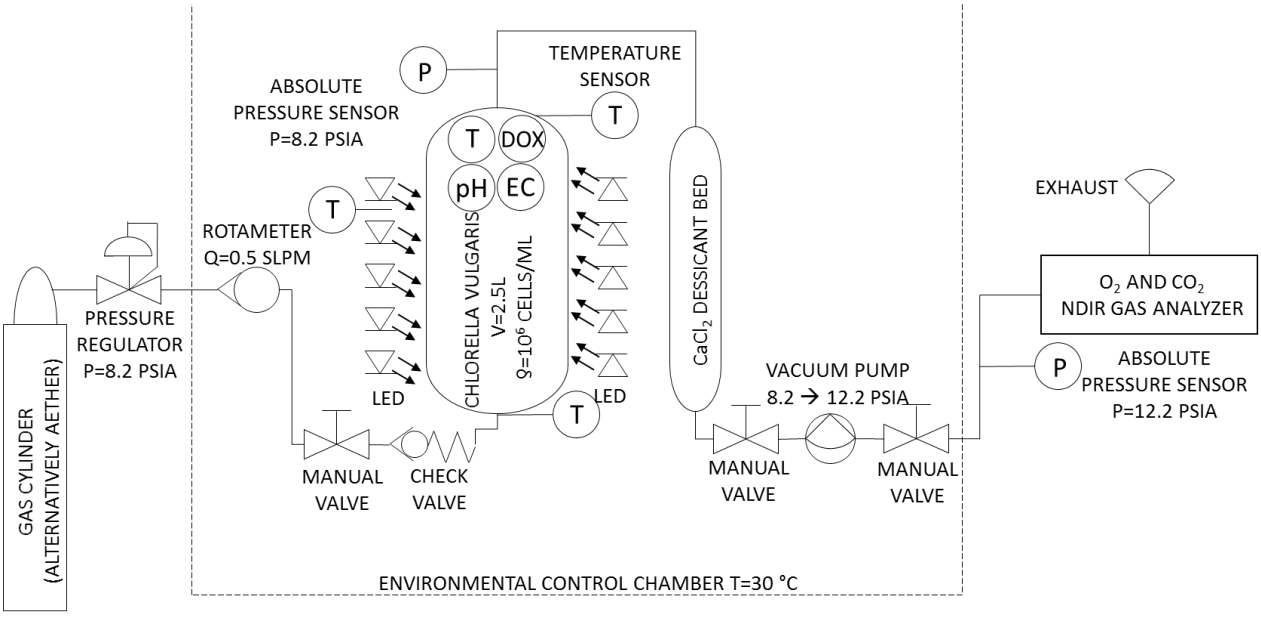

Figure 5: Piping and Instrumentation Diagram of test setup

The required air composition can either be achieved by a custom premixed gas cylinder or by the use of mass flow controllers mixing individual supply cylinders. Consistent pressure and flow rate are achieved with pressure regulators and rotameters. A check valve together with a ball valve, immediately below the reactor, both prevent the gravity-driven flow of algal solution through the pipes in case the air flow is stopped or the photobioreactor gets detached. Besides exchanging gases between the gaseous and the liquid phase, the bubbles also fulfill the purpose of mixing the algal solution within the reactor to achieve a uniform distribution. Furthermore, a magnetic stir bar helps to prevent cells from settling on the bottom of the reactor.

The top of the photobioreactor has an outlet connection that allows the air of the reactor's headspace to escape. Metal tubing guides the air to the bottom of a calcium chloride ($CaCl_2$) desiccant bed. This granulate dries the gas stream with a humidity content of about 80 % by absorbing water molecules. After leaving the desiccant bed, the gas

is pulled out by a diaphragm vacuum pump. Here, the pressure is changed from the desired set point back to atmospheric conditions of about 85 kPa in Boulder. The vacuum pump is necessary upstream of the gas analyzer, to provide the required ambient conditions for precise measurements. The exhaust stream from the gas analyzer, is subsequently vented to the ambient environment.

To allow for intermediate sampling of the algal culture without disturbing the pressure environment within the reactor, a sample port was implemented on the bottom of the photobioreactor. A needleless injection site maintains the seal of the reactor that gets mechanically opened once a syringe is attach to the injection port. Using the syringe piston as a driving source against the under- or overpressure, a 3 ml algal sample can be taken. Additionally, the port serves the purpose of refilling the algal photobioreactor with small quantities of growth medium to adjust for the sinking water level due to evaporation.

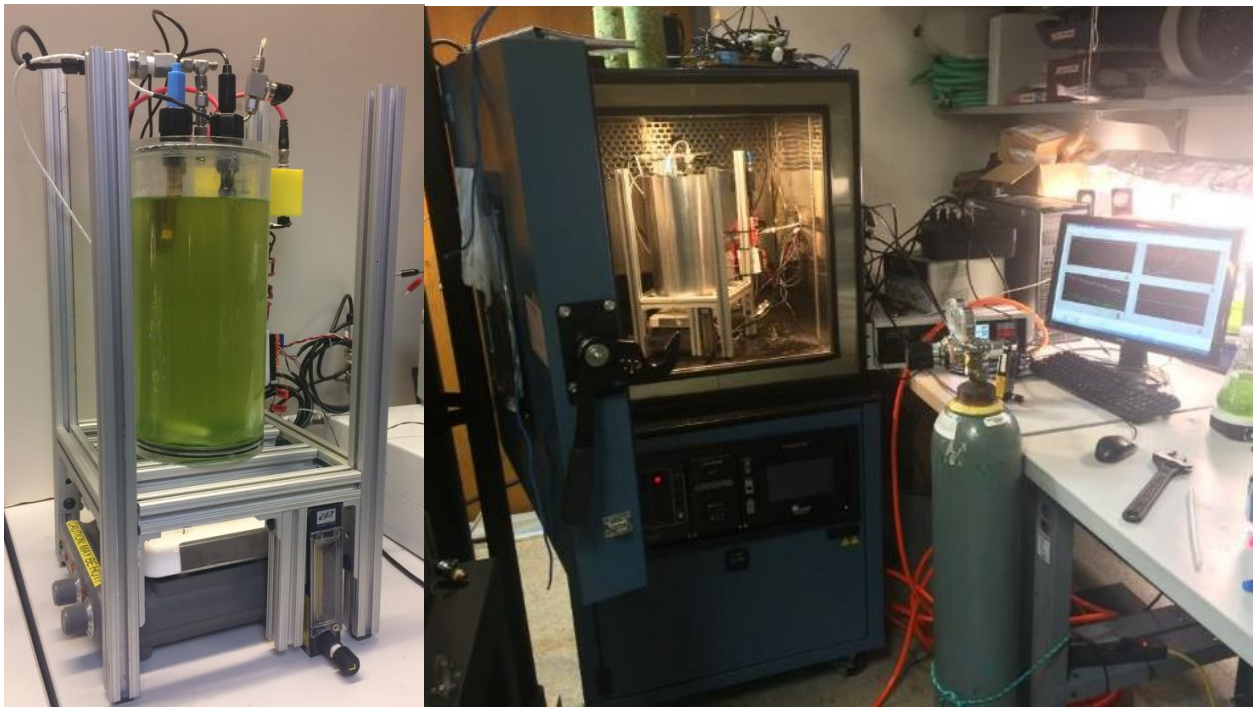

Figure 6: Experimental test setup

The leak rate of the entire system was determined to be $1 \cdot 10^{-4}$ Pa·m^3/s and was verified by calculation to be sufficiently slow for accurately measuring algal metabolism. It is important to verify the leak rate of the system before each test, as several seals need to be opened for cleaning purposes everytime the photobioreactor is loaded. A procedure was developed that allows for assembly of the photobioreactor and subsequent leak test without loaded algae. Once the test specific leak rate of the system is confirmed, the reduced pressure in the system can be used to easily inject the algal solution from a blood plasma bag.

Table 3: Sensor specifications

Sensor	Model	Manufacturer	Range
Temperature (l)	KIT-301	Atlas Scientific	-126.000 to 1254.000 ± (0.10 + 0.0017·T) °C
Temperature (s)	103AT-4-70261	Semitec	-50 to 110 ± (0.01·T) °C
Absolute pressure	PX309-030A5V	Omega Engineering	0.00 to 30.00 ± 0.075 psia
pH	KIT-101P	Atlas Scientific	0.001 to 14.000 ± 0.002
DOX	KIT-103D	Atlas Scientific	0.01 to 35.99 ± 0.05 mg/l
Conductivity	EC-KIT-0.1	Atlas Scientific	0.07 to 50,000.00 ± (0.02·EC) µS/cm
Oxygen	ZRE NDIR/O2	CAI	0.00 to 25.00 ± 0.13 %
Carbon dioxide	ZRE NDIR/O2	CAI	0.000 to 1.000 ± 0.005 %

2. Sensor design

The entire setup is equipped with sensors to measure algal metabolism and environmental conditions. Locations of the sensors are shown in Figure 5 with details of the sensors provided in Table 3. All sensors interface either via analog signal or serial communication to the computer and are received, processed, and displayed via LabVIEW. The program also logs the data in a Technical Data Management Streaming (TDMS) file that can be read by analysis tools like Microsoft Excel or MATLAB. The program also controls the light cycle of the LED lighting via an analog output and a solid state relay.

There are 4 probes fed through the top lid of the photobioreactor which can be seen in Figure 6. They measure pH, dissolved oxygen (DOX), conductivity, and temperature directly in the algal solution. The absolute pressure is recorded directly at the outlet of the reactor. Additionally, an absolute pressure sensor is installed at the inlet of the gas analyzer, as the measurements of the gas analyzer are pressure dependent. The gas analyzer measures carbon dioxide and oxygen concentration through a nondispersive infrared (NDIR) sensor and an electrochemical fuel cell.

D. Challenges

In a flow-through setup, such as presented in this work, the accuracy of the sensors plays a critical role in measuring the algal metabolism. Due to the continuous gas flow and limited photobioreactor size, the rate of change in gas composition is smaller than in closed loop systems that accumulate variations over time. Initially, it was thought that the most accurate results would be obtained directly in the gas phase of the photobioreactor. However, tests that used small scale sensors showed that a resolution of 1000 ppm gas concentration and uncontrolled effects due to humidity, pressure, and temperature prevent the collection of useable data. The photobioreactor was hence updated to accommodate a gas analyzer with a resolution of 10 ppm. Due to the experimental configuration and size of the anaylzer, however, only the exhaust gas from the photobioreactor can be measured. Through the vacuum pump and gas dryer unit, the gas stream is pretreated to match the gas analyzers small input gas parameters in terms of humidity and pressure. The continuous flow also demands low leak rates below $1 \cdot 10^{-4}$ Pa·m^3/s in the system to allow the precision needed for the gas measurements.

Part of the challenge in achieving high resolution gas measurements is the provision of dry gas to the gas sensors. Drying the air stream is commonly done with silica gel (SiO_2) or Drierite. However, the silica gel also absorbs carbon dioxide, which later gets released again when the desiccant bed becomes saturated with water. As the carbon dioxide produced by the algal cells is measured, this secondary reaction in the dessicant bed falsifies the results. This observation has previously been reported in the research community[27]. To avoid this problem, calcium chloride ($CaCl_2$) was selected. Measurements can also be inadvertently affected due to secondary reactions in the photobioreactor. The solubility of carbon dioxide and oxygen in water is, for example, dependent on the temperature. A temperature fluctuation of less than 1 K therefore has to be maintained in the algal solution to adequately miminize this effect.

Cell counts are performed on a small sample and then extrapolated to the entire photobioreactor volume. In order to achieve a representative sample, the algal solution is required to be well mixed without any settlement or biofilm formation on the walls. In order to avoid biofilm formation, the surface finish as well as the materials have to be taken into account. Porous surfaces, as found in diffuser stones and materials, such as Teflon, attract algal growth on their surfaces and have to be avoided.

Finally, since the experiment durations are on the order of days to weeks, accessibility to the test setup without disturbing the environmental conditions in the photobioreactor is crucial for maintenance or repair needs. This was achieved by isolating the actual photobioreactor volume from the electronics, including lighting, pressure, and temperature measurements. The only wetted parts that are crossing the pressure containment of the photobioreactor are the sensor probes that are directly measuring characteristic in the liquid phase.

III. Results and discussion

In order to experimentally characterize the testbed physical performance, a photobioreactor filled with distilled water was run for 40 hours under the desired test conditions. The input gas stream consisted of air enriched to 0.1 % carbon dioxide and was fed into the photobioreactor with a constant water temperature of 30°C. This test was conducted under a reactor pressure of 85 kPa. It was verified that the day/night cycle did not induce a secondary effect on the measurements, as can be seen from the constant values in Figure 7 (left). In addition to this control test, a pilot test run with *Chlorella vulgaris* was conducted for 72 hours under otherwise identical conditions. It can be seen that the metabolism changed in response to the light activity cycles over the duration of a day in Figure 7 (right). Comparing the control to the test showed that the measured changes are due to the algae and not due to the altered temperatures which would cause altered solubilities of the gases in the liquid phase. Within an hour of light activation,

an increased oxygen evolution can be observed, which is maintained throughout the daylight period. Both the dissolved oxygen as well as the oxygen concentration in the gas stream show this trend. Carbon dioxide levels can be seen to decrease during the day due to increased fixation rates, with pH consequently increasing as result of the dissolved carbon dioxide. Future tests will be conducted to assess the repeatability of the pilot test results obtained in this photobioreactor system.

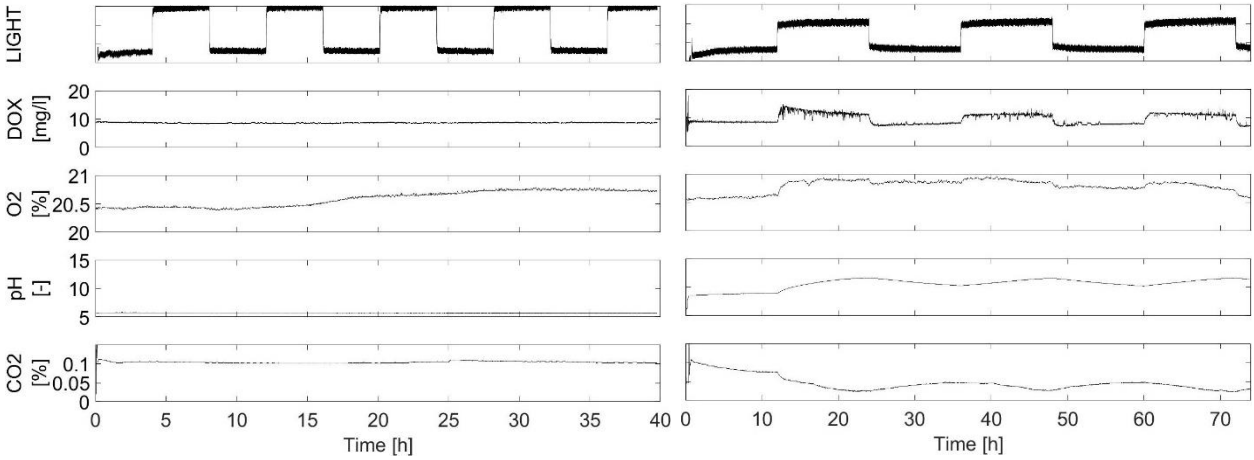

Figure 7: Reference measurements of water filled photobioreactor (left) and measurements of algal filled photobioreactor (right)

The sample port allows for further analysis such as absorptivity measurements using a photometer and cell counts on negligible small samples. Cell absorptions where performed at 450, 600, and 690 nm. A trade between sensitive and robust measurements at wavelengths at the absorption centers (450 and 690 nm) and between them (600 nm) has been previously suggested.[28] Analysis of the three absorptivities tested in this experiment has shown close correlation between them. Therefore, a sensitive wavelength of 450 nm that closely matches the linearity of the Beer-Lambert law was selected for future experiments.[28] Figure 8 shows the measured growth curve of *Chlorella vulgaris* in the newly developed photobioreactor. It resembles a typical algal growth curve consisting of a lag phase during the first day, followed by an exponential phase from day 1 until day 5 and, finally, transitioning to a saturation phase from day 5 on. The cell density at the start was $1 \cdot 10^6$ cells/ml and increased to a maximum of $1 \cdot 10^8$ cells/ml with a typical cell viability above 95 %. During the maximum growth rate, a doubling time of 20.9 hours was achieved. The pH of the algal solution stayed relatively constant, between 9 and 11, during the entire test duration without active control. Salinity, pressure, and water temperature measurements were used as verification of sufficient nutrient availability as well as constant pressure and water temperature during the experiment.

Different environmental conditions, such as pressure, will affect the algal metabolism. The output carbon dioxide and oxygen concentration, the cell densities, and the growth rate will be used in future studies to characterize the effect from a system perspective. To measure the carbon dioxide fixation and oxygen evolution rate, recorded output carbon dioxide and oxygen concentrations will be subtracted from the known input concentrations. The integrated values for total oxygen evolution and carbon dioxide concentration can then be compared to the absolute cell number increase for establishing a relationship between algal metabolism and biomass production.

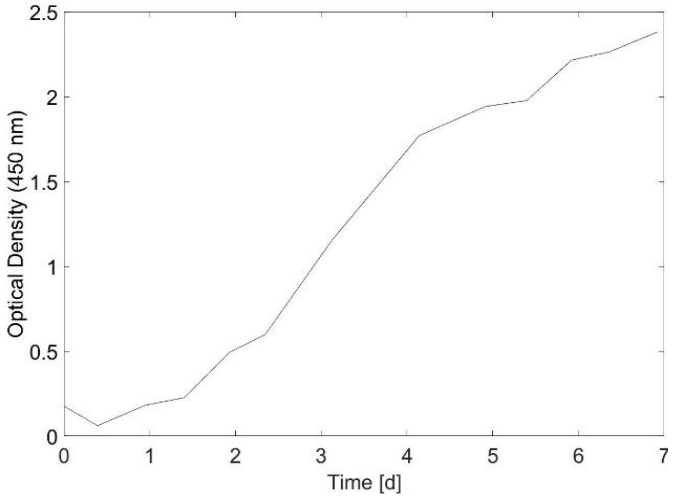

Figure 8: Growth curve of *Chlorella vulgaris* cultivation in photobioreactor for 7 days at 450 nm.

IV. Conclusion

This work served to design, develop, and evaluate an experimental photobioreactor, through multiple iterations, that is capable of operating within the typical environmental conditions anticipated for future space habitats. The specific interest lies in the alteration of pressure and gas composition to characterize algal performance under NASA's proposed exploration atmosphere. It was experimentally demonstrated that the algal photobioreactor can achieve growth rates and cell densities comparable to typical benchtop bubble column photobioreactors. A baseline experiment demonstrated that no confounding artifacts are introduced from the test setup itself and its variation over time, such as the day/night cycle. Challenges, including altered gas solubilities at different temperatures, low leak rates, and accessibility, were shown to be successfully handled. Measurements made in the gaseous stream exiting the photobioreactor record the system performance continuously throughout the experiments. Point measurements, using the sample port, can be used to measure cell density and from that, algal behavior can be inferred. Both data sources together can be used to characterize algal bioreactor performance, using relevant characteristics such as oxygen evolution and carbon dioxide fixation rates per cell. The collected data increases the understanding of algal growth under reduced pressure and provides the basis for future overall system trade studies needed to implement an algal photobioreactor in a spacecraft. Even though primarily intended for conducting studies under reduced pressure, this setup can also be used to evaluate algae cultures under a variety of other conditions of interest for spaceflight applications, as well as for related terrestrial purposes.

Acknowledgments

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