PBR@LSR: the Algae-based Photobioreactor Experiment at the ISS – Operations and Results

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The experiment Photobioreactor at the Life Support Rack (PBR@LSR) was launched to the International Space Station (ISS) in the second quarter of 2019. The objective was to prove the feasibility of a xenic long-term cultivation of microalgae (*Chlorella vulgaris*) under space conditions and to demonstrate for the first time the technology and performance of a hybrid life support system (combining physicochemical and biotechnological components). The experiment and development of the PBR was initiated in 2015 by the German Aerospace Center (DLR) and the Institute of Space Systems (IRS) of the University of Stuttgart with Airbus Defence and Space as prime for the flight hardware. The experiment on the ISS, as well as a parallel experiment at IRS, started in June 2019. An unexpected and not yet fully understood lack of power supply ended the experiment earlier than planned - after two weeks of operations. The samples of those first weeks came down with the SpX18. Analysis and maintenance on board did not allow the identification of the power supply failure cause. This paper will summarize the operational phase and the results obtained during it, as well as the on-going failure investigations.

Nomenclature

ACLS	=	Advanced Closed Loop System
DLR	=	German Aerospace Center (Deutsches Zentrum für Luft- und Raumfahrt)
DSN	=	Diluted Seawater Nitrogen Medium
EC	=	Experiment Compartment
EST	=	Experiment Sequence Test

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FPA	=	Flat Panel Airlift
IRS	=	Institute of Space Systems (Institut für Raumfahrtsysteme)
ISS	=	International Space Station
LE	=	Liquid Exchange
LiED	=	Liquid Exchange Device
LSR	=	Life Support Rack
LSS	=	Life Support System
MDL	=	Mid-deck Lockers
PBR	=	Photobioreactor
PBR@LSR	=	Photobioreactor at the Life Support Rack
rh	=	Relative humidity
UOC	=	User Operations Center
WRS	=	Water Recovery System
µg	=	Microgravity
CO_2	=	Carbon dioxide
O_2	=	Oxygen
H_2O	=	Water

I. Introduction

Future human space flight missions in the coming decades should bring humans beyond Low Earth Orbit, with current mission scenarios such as a base on the lunar surface or a mission to Mars.[1-3] These scenarios will require systems, including the Life Support System (LSS), as independent from Earth's resources as possible. The increased duration and the farther distance from Earth, will make it difficult if not impossible to consider resupply missions as often as we currently have at the International Space Station (ISS). On board the ISS, 42% of the oxygen (O_2) is recovered from the carbon dioxide (CO_2) produced by the astronauts. For this process a Sabatier reactor and water electrolysis are used. An extra amount of water is still brought from Earth, in order to produce the rest of oxygen required. Nearly 90% of the service/domestic water (H_2O) is recycled by the Water Recovery System (WRS).[4] All these -functions can be achieved by technologies using physicochemical principles (state of the art physicochemical processes), which are currently not able to produce food. For the ISS it has up to date been always produced on Earth and transported up to the station. The food production requires the use of bioregenerative technologies which at the same time can represent an advantage for the further closure of the oxygen cycle. The biological systems could in the future complement the already in use physicochemical ones.

A potential candidate to be used for biological technologies are microalgae, which like higher plants carry out photosynthesis, thus using carbon dioxide, water, nutrients and light to produce oxygen, water and biomass. Some of their advantages over higher plants are their higher harvest index, higher biomass productivity, a more efficient light utilization and lower water requirement.[5-6] The drawback of microalgae, compared to higher plants, is that they can represent a maximum of 35% of the human daily food consumption to ensure an equilibrated diet, due to its high content of proteins.[7] Thus, algae can supplement a diet, which can be composed by higher plants in-situ produced biomass and/or prepackaged food from Earth, depending on the mission scenario. The use of both higher plants and algae for space applications has been studied for decades, and several experiments both on Earth closed environments and in space have already taken place.[8-9]

At the Institute of Space Systems (IRS) at the University of Stuttgart (Germany), the LSS research has been focused on the use of microalgae for over a decade. Starting with a trade-off of potential candidate species, *Chlorella vulgaris*, a spherical single cell organism with a mean diameter of 6 µm[10], was selected for its robustness, providing a high resistance to cross contamination[11], and being able to grow in a wide range of CO_2 concentrations[12], pH and temperature levels[13]. Over the last years Flat Plane Airlift (FPA) Photobioreactors (PBRs) have been used at IRS to gain experience in the long-term, efficient and non-axenic cultivation of *C. vulgaris*. Biology itself is not the only focus, but also the engineering effort required for the infrastructure design.[14-15] In 2015 the gained knowledge served as a base to start the development of the µg-adapted PBR experiment – Photobioreactor at the Life Support Rack (PBR@LSR) – initiated by DLR and the IRS, with Airbus Defence and Space as prime for the flight hardware. The PBR experiment flew to the ISS in 2019, with an operational phase shorter than expected due to a power loss failure. This paper presents the operation phase and the results obtained up to date.

II. PBR@LSR Experiment

The PBR@LSR experiment's main goals were to demonstrate the functionality and feasibility of a hybrid LSS approach, the short and long-term photosynthetic conversion of concentrated CO_2 into O_2 and biomass and to prove the stability of an algae system in space. To fulfill these goals a microgravity-compatible PBR chamber was designed and the cultivation parameters and techniques to cultivate for 180 days were set. To demonstrate the hybrid LSS approach, the PBR was meant to be connected to the Life Support Rack (LSR-formerly known as ACLS)[16], a physicochemical LSS technology which was installed in the ISS in 2018. Figure 1 shows the processes carried out by the LSR: CO_2 extraction from the cabin atmosphere, CO_2 processing in a Sabatier reactor, and O_2 production by water electrolysis. A surplus of the extracted CO_2 could be directed to the PBR, providing the hybrid connection, and the produced O_2 added to the ISS atmosphere. For this experiment, a regular biomass extraction was planned, which in the future could potentially be used as a food supplement source. A back-up CO_2 bottle was foreseen to ensure the experiment could take place, in case the LSR is not able to deliver CO_2.

The experiment contained a µg-adapted PBR to cultivate the green algae *Chlorella vulgaris*. The reactor was contained in a gas-tight Experiment Compartment (EC), where several sensor and actuators were in charge to keep a proper cultivation environment (gas concentration, temperature, lighting). The only crew interaction required during the experiment were the so-called Liquid Exchange (LE) activities, to be carried out every two weeks during the entire operational phase. This included the initial inoculation, the feeding and harvesting during the experiment and the sample collection.

To evaluate the experiment, data from the sensors as well as the downloaded samples were foreseen. The sensors shall provide information of the performance of the PBR. The samples, stored and downloaded for further analysis on Earth, shall provide relevant information of the influence of µg and radiation effects.

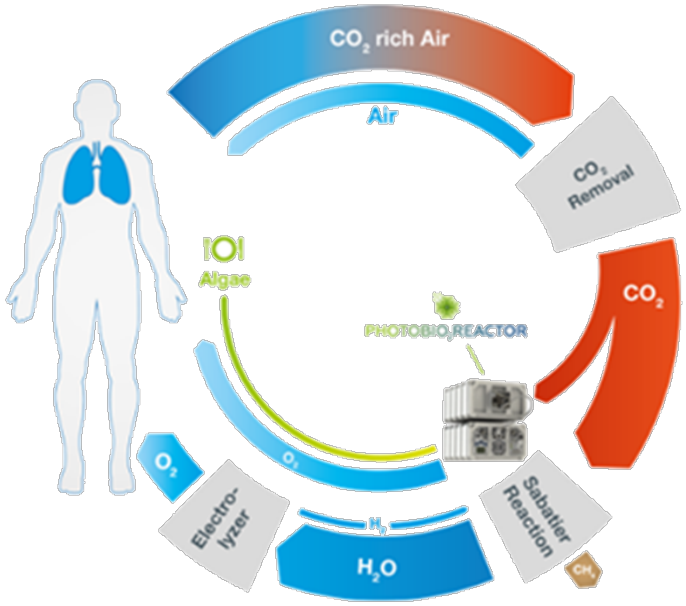

Figure 1. Process schematic of the LSR with the added PBR experiment, a hybrid system approach

The required hardware for the experiment consisted of the experiment itself, Figure 2, the connection to the LSR, Figure 3, and the LE equipment, Figure 4. The experiment consisted of two Mid-deck Lockers (MDL). The lower MDL contained the EC, the required electronics and other elements such as a CO_2 pulse chamber and a humidity absorber. The upper MDL contained storage for the Liquid Exchange Device (LiED) and the back-up CO_2 gas bottle. The connection to the LSR provided the required tubing. It was also equipped with a flow restrictor (providing a maximum flow rate of 1 g/day) and a check valve, to ensure no air from the PBR flows to the LSR. A ready-signal could inform if the LSR facility was working and able to provide CO_2, allowing the PBR to activate the back-up bottle if required. The LE equipment included the LiED, dedicated syringes, sample return bags, gloves and cleaning wipes. The syringes and the sample return bags containing the required substances needed to be stored in cold stowage.

Further details on the development and preparation of the PBR@LSR experiment as well as on a successful long-term cultivation of *Chlorella vulgaris* in the given µg-capable cultivation system on Ground with high biomass turnover can be found in several publications. [17-29]

Figure 2. PBR@LSR experiment

Figure 3. PBR-LSR CO_2 interface

Figure 4. Liquid Exchange equipment.
Training model

III. Operational Phase

The PBR@LSR operations took place under Airbus Defence and Space responsibility with support from IRS and were coordinated with DLR, ESA and NASA. The same up/down data link as for LSR was used.

A. Pre-launch

The experiment operations preparation phase started a couple of months before the launch to ISS, with the preparation of the algae, scaling-up from *C. vulgaris* strain SAG 211-12 single cells to a suspension culture in a 6 L reactor, Figure 5, which took place in the dedicated laboratory at IRS. The algae were not inserted directly in the reactor on ground, but into syringes, until inoculation took place on board the ISS.

A couple of weeks before the start, all required syringes were filled, with the prepared algae, but also with start medium, nutrients and termination solution. The sample return bags were filled with fixative. The reactor itself, until the start of the experiment, was filled with sterile water. The algae could be first inoculated after a high concentrated DSN-medium was inserted and properly mixed. The termination syringe included an inhibitor for photosynthetic and cellular respiration processes as well as an antibiotic/antimycotic solution. The different labeled syringes are shown in figure 4. The sample return bags included 20 ml of a cryofixation solution (PVP concentrate).

The syringes, Figure 6, were delivered by IRS 11th April 2019, two weeks before the planned date for the SpX-17 launch. The transport of the syringes to the launchpad, where the rest of the equipment was already present, occurred at 4°C and in darkness.

B. Launch

The launch of the experiment on board of SpX-17, took place, after some delay, the 4th May 2019, Figure 7. The delay was shorter than the life expectancy of the dedicated syringes. Thus, a refurbishment of the liquids in PBR Syringes was not required. SpX-17 berthing took place two days later, Figure 8. Once in orbit all filled syringes and bags were stored at 4°C.

Figure 5. Subitec® Reactors at IRS-lab, used for the scale-up process

Figure 6. PBR@LSR Syringes, ready for transportation

Figure. 7. SpX-17 Launch. Credits SpaceX

Figure 8. SpX-17 mission arriving to the ISS. Credits NASA

C. On-orbit

The experiment was successfully installed in the EXPRESS Rack Locker in the US Destiny Lab, as it can be seen in Figure 9, at GMT 2019/133, and started at GMT 2019/157, Figure 10.

To start the experiment, the volume of two syringes of start medium were first inserted, followed by a mixing time of two hours. This procedure, tested on ground, ensured a proper mixture of the highly concentrated medium from the syringes and the water included in the reactor. After that, the amount of two algae syringes were satisfactorily inserted, providing within the reactor an estimated initial experiment concentration of 1.2 g/L. During this inoculation activity, a first sample was taken, from a separate alga syringe. This sample shall allow in further analysis the evaluation of potential effects of the storage and transportation, providing the status of the algae right before inoculation in the PBR. Before insertion in the system the syringes were stored at 4°C and in darkness for 8 weeks. This storage scenario was tested on Earth several times prior to the ISS experiment.

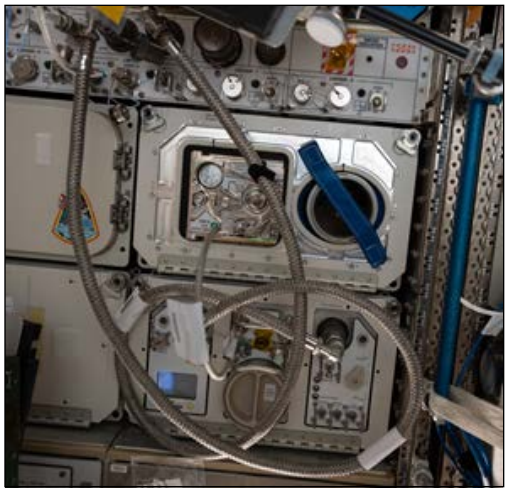

Figure 9. PBR@LSR Installed in the ISS US Destiny Lab. Credits NASA

Figure 10. Start Medium insertion using the Liquid Exchange Device at GMT157. Credits NASA

The connection with the LSR was not performed, since the LSR was not fully operational but still in commissioning phase. The back-up bottle was, therefore, the only CO_2 source.

The nominal operations of the experiment, as shown in Figure 11, had foreseen an experiment duration of six months, with a LE crew activity every two weeks. For each activity the insertion of two nutrient syringes was planned, while extracting simultaneously the same amount of algae suspension from the experiment. In most cases this extracted suspension was meant to be trashed, with the exception of several samples, distributed over the experiment time: after 2 and 4 weeks and after 3 and 6 months.

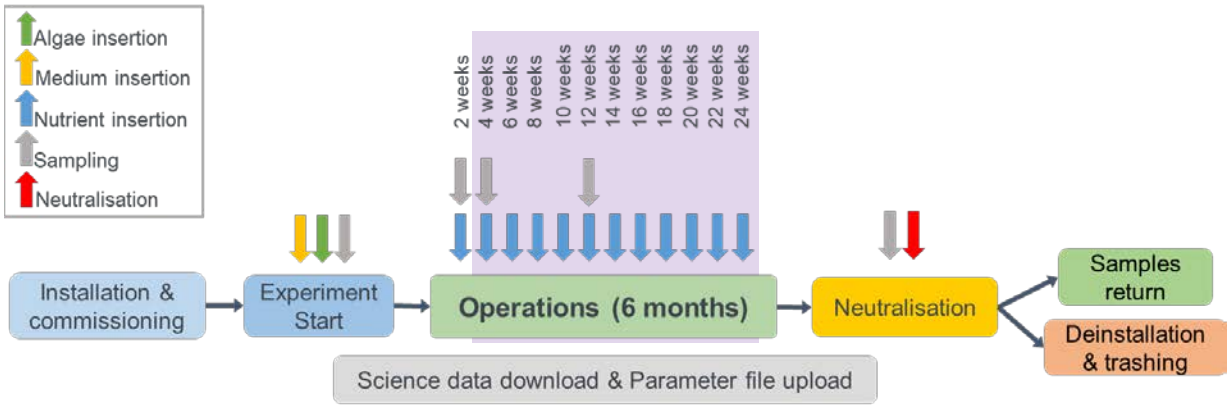

Figure 11. Planned Operational scenario for the PBR@LSR experiment. *The purple area shows the LE and samples that could not take place due to premature experiment termination*

The first LE after inoculation took place as planned at GMT2019/171. Figure 12 shows the LiED after the second nutrient syringe was inserted in the PBR. On the right side, the nutrient syringe (blue stamp) recently emptied, and on the left side a sample syringe (grey stamp), with the extracted produced biomass, which was then transferred to a sample return bag. During this period, the sensors' data was saved several times a minutes. The data could successfully be accessed in regular intervals through the UOC at Airbus Friedrichshafen (ESC-FN ops center).

One day after, at GMT 2019/172 a sudden lack of power in the experiment caused all sensors and actuators within the PBR to stop working. After several recovery attempts the failure source could unfortunately not be identified, and the experiment was prematurely terminated. Several external failure modes were discarded, after for example an

exchange of the power supply cable. A search of internal failures was not possible, due to the impossibility of maintaining the experiment on board.

In order to get the most science data of the experiment, despite the premature termination, the three remaining samples bags were used to sample the two back-up algae syringes. They had been stored at 4°C and in darkness for 11 weeks in microgravity, after the 4 weeks of transport and storage on ground. The third sample bag was used to extract a sample of the reactor after several weeks of power-off, to evaluate how the culture inside the reactor had evolved under space conditions when the foreseen cultivation environment (required light, movement of the algae suspension, gas management) was not provided.

The sample return bags, figure 13, returned back to Earth with SpX-18, 27th of August 2019, and are currently being processed at IRS for the post-flight analysis. The sample bags have been stored since sampling until its analysis at a temperature below -20°C. The experiment itself is currently stored at the ISS.

Figure 12. LE activity at week 2, GMT 2019/171.
Credit: NASA

Figure 13. Sample Return bag, filled with *C. vulgaris* after cultivation at the ISS.

D. Post-flight analysis

The post-flight analysis, currently taking place, includes the processing of the sensors data and analysis of the return samples. Sensors data analysis includes the processing of the O_2 and CO_2 concentration, temperature, relative humidity, pressure, pH and biomass. The samples post-flight analysis strategy was planned by the science team at IRS based on the experience with ground experiments. The post-flight analysis shall provide information of the influence of µg and cosmic radiation at different levels. Several partners are involved, since they can provide analysis tools and equipment not available at IRS lab. Both tasks are currently in progress and are expected to be finished during 2020. A preliminary discussion of current results is presented in Chapter IV.

E. Parallel Experiment

A parallel experiment was run at IRS, reproducing all the events occurred on board the ISS. The parallel experiment was run with a shift of one week with respect to the ISS one, to provide enough margin to reproduce any unexpected events on ground.

A breadboard, previously used for two long-term experiments (>180-days) and the Experiment Sequence Test (EST) had been refurbished for this parallel experiment. Thus the model used was not one to one with the flight model, but replicated the same functionality and experiment conditions as well as the exact same materials for the algae suspension loop.

IV. Current Results

The post-processing of the experiment run and its results is still an ongoing process. The three main focusses are on the data produced by the sensors, the analysis of the samples and the failure analysis. These three aspects are crucial to understand the strengths and weaknesses of the experiment and to identify lessons learned for future experiments.

The experiment ran for about 15 days. The sensors data was downloaded some hours before the loss of power. Once the experiment had no more power, it was not possible to download further data. Thus the last hours of data are currently not available. During the first week the experiment experienced some unexpected system restarts. This first restarts brought the experiment back to stand-by modus, which implies no lighting or pump movement in the systems. This restarts can be observed in Figure 14, in the four temperature drops (under 20°C), since during these periods the highest source of heat, the LED panels were off. The system was brought back to nominal from ground after each restart. Since there was no continuous communication between the experiment and ground, there was a time gap between identification and restoring nominal operations. These periods were shorter than the 48-hours power-off periods tested on the breadboard phase, which showed no major influence in the further cultivation of *C. vulgaris*. The problem was resolved, and no restarts were experienced during the second week.

A. Sensors Data

The data from the sensors was downloaded regularly and processed during the experiment. The sensors showed that temperature and humidity regulation worked properly, and were kept within the expected ranges. Figure 14 shows the evolution of those parameters. For the ISS Experiment, the relative humidity (rh) was between 48.6 and 76.4 % and temperature ranged from 18.2 to 24.3 °C. For the parallel experiment rh was between 49.6 and 70.0 % and temperature ranged from 22.3 to 27.1 °C. The pH level stayed within the expected range, with values from 6.3 to 8.1 for the ISS experiment and 6.0 to 8.5 for the parallel experiment.

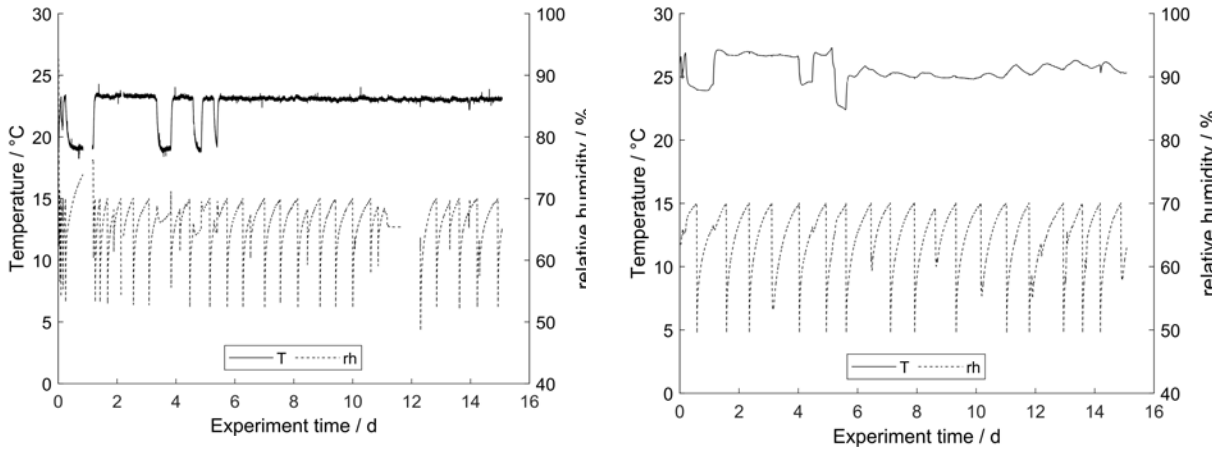

Figure 14. Temperature and relative humidity levels at ISS Experiment (A) and Parallel Experiment (B).
For the flight experiment the missing points are missing data from the power-off events and in case of the rh data also sensor problems that occurred between day 11.6 and 12.3.

The biomass concentration data was scattered, which shows homogeneities present in the alga loop, which could be caused by circulating bubbles or biomass agglomerations. The data has been filtered for a RSD of 1%. Figure 15 shows the evolution of the OD / Biomass concentration both for the ISS experiment and the parallel one. Comparing the parallel experiment on ground with the flight experiment, it can be observed that in general higher biomass densities were measured during the first 10 days. This effect can be caused by the fact that on ground high sedimentation was observed and the sensor is only able to measure in the mobile phase of the algae suspension. Even if it is not possible to quantify it, it can be assumed that much less sedimentation and potentially adhesion occurred in the ISS experiment. Due to this effect, it is not possible to identify with the biomass sensor data if the algae growth itself is affected by microgravity conditions. If the experiment would have run nominally, the total biomass at the end of the ground experiment could have been measured after opening the reactor. This would have allowed the estimation of sedimentation on ground. The same measurement could not have been done in space, since the experiment was planned to be trashed, thus not returning to Earth for further analysis. However, a correlation of the gas consumption/production, the algae growth measured in the liquid phase both in space and on ground, and the total

biomass in the ground could have been used to compare the amount of biomass in both systems. On the ISS experiment, a biomass density increase can be observed up to day 10. At this point the density starts decreasing. This could be explained by a possible acute lack of required nutrients at this time. A biomass loss in the mobile phase up to day 14 is the consequence.

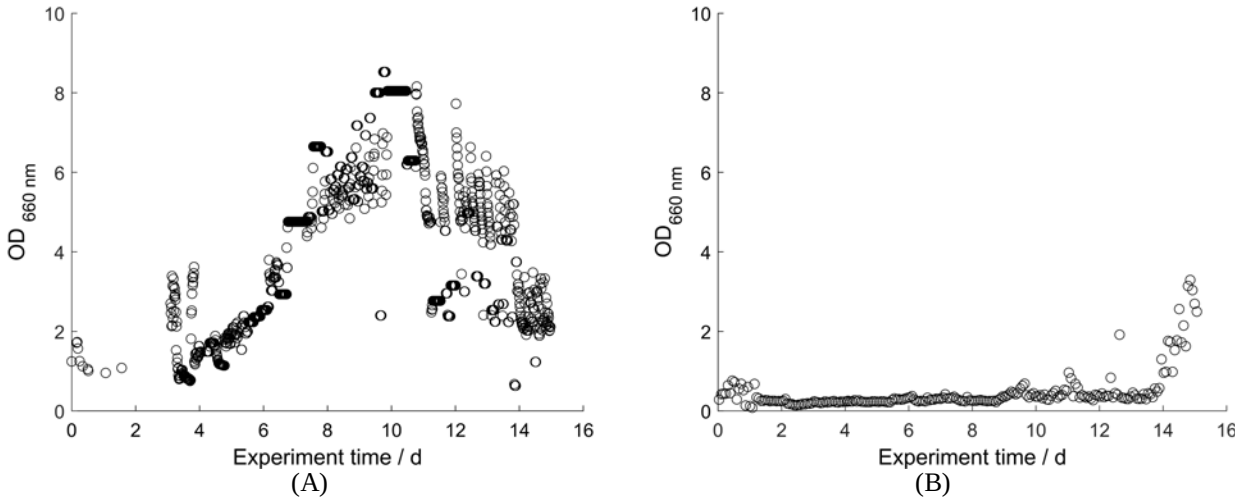

Figure 15. Biomass Sensor data at ISS Experiment (A) and Parallel Experiment (B).
Data filtered for RSD 1%.

O_2 and CO_2 concentrations showed an unexpected behavior, which suggests a potential leakage from the EC to the O_2 absorber compartment. The oxygen level decreased rapidly within the EC, going from levels of 12% to close to 0% in about half a day. This decrease clearly suggests that the O_2 absorber was partially working, even when the absorber was supposed to be off. Tests in the laboratory during the breadboard phase showed that the absorber also absorbs CO_2 particles. In nominal operations that was not a problem, since the absorber was only activated after cabin flushing when there was almost no CO_2 in the EC and for short periods of time, which could be processed during the post-flight data analysis. Therefore, the CO_2 consumed could easily be estimated without the influence of the O_2 absorber. However, since the absorber was partially working constantly, part of the CO_2 introduced in the EC was constantly being absorbed as well. A characterization of the CO_2 absorbance in the O_2 absorber in this particular scenario is not possible, since several parameters such as degradation are highly dependent on the flow path, which cannot be defined, since the absorber was not operating in a nominal state but was absorbing through an unknown leakage.

B. Samples Analysis

In total 10 samples had been taken, five from the ISS Experiment, five from the parallel experiment at the IRS lab. The preparation of the samples has already taken place. The samples have been thawed, the PVP has been removed and individual samples for the analysis have been prepared. The following analysis are currently undergoing, and results are expected in the coming months:

- Biotechnological analysis: Influence of µg and cosmic radiation on growth dynamics as primary marker for culture functionality
- Physiology (N and P uptake rates, photosynthetic performance, biomass quality (pigments / proteins),: Influence of µg and cosmic radiation on extra- / intracellular mass transport and biomass
- Cell and culture morphology: Influence of µg and cosmic radiation on the "appearance" of the culture, which can influence the flow and cultivation properties.
- Genetic testing of marker genes: Investigation of the influence of µg and cosmic radiation on selected *C.vulgaris* genes. These are intended to provide information about possible damage to the genome from space use.

- Microbiome: Investigation of the influence of µg and cosmic radiation on the accompanying bacterial and eukaryotic microbiome of the used non-axenic *C. vulgaris* culture
- Proteome: Investigation of the influence of µg and cosmic radiation on key proteins for the functionality of *C. vulgaris*

C. Failure Analysis

At this moment the only conclusion that can be taken from the last downloaded sensors data is that the algae and the reactor chamber were working properly. Even if the O_2 production and CO_2 consumption could not be evaluated, the biomass sensor clearly shows a biomass production inside the reactor. The reactor chamber was providing the required support for the algae-loop, since up to this point, the integrity of the membrane can be assumed. In case of a membrane rupture the humidity levels within the experiment chamber would have raised - as observed in previous experiments in the IRS laboratory. The failure issue is, thus, likely to be caused by a technical issue, not by the biological material included in the experiment. Further investigations will take place in the coming months.

V. Conclusion

Biotechnological systems might complement the current physicochemical LSS technologies for future human space flight missions, where the increased mission duration and distance from Earth will make it necessary to be as independent as possible from Earth's resources. A potential candidate for a biotechnological technology are microalgae.

The experiment PBR@LSR, a DLR – IRS – Airbus DS project, was developed to test the long-term cultivation of microalgae in space. Its goal was also to demonstrate for the first time a hybrid system, combining a biotechnological and physicochemical system, the LSR.

The experiment was launched to the ISS in May 2019 and ran for only two weeks. A power loss caused a prematurely termination of the experiment, which was planned to run for 180 days.

The data from the sensors has been analyzed. The data shows that temperature and humidity regulation worked as planned. The pH level stayed within the expected values. Unfortunately, O_2 production and CO_2 consumption cannot be evaluated, since both substances were continuously being absorbed, due to a leakage in the O_2 absorber compartment. The biomass sensor data shows a continuous growth during the first 10 days, which is considerably higher than on the parallel experiment. A higher growth was expected to be observed in the liquid-phase measurement in the beginning of the experiment, since in microgravity no sedimentation and potentially less adhesion would occur.

Several samples from the experiment were sent back to Earth and are currently under study, to investigate the influence of µg and cosmic radiation on biotechnological aspects, its physiology and morphology, among other aspects.

Due to the shorter duration of the experiment, it was not possible to fulfill the experiment goals. However, with the currently available experiment data, it can be assumed that the interruption of the experiment was caused by a hardware malfunction, not related with the biology itself. Further research is thus required to prove the long-term feasibility of a micro-algae based LSS component in microgravity.

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