The final configuration of the algae-based ISS experiment PBR@LSR

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The spaceflight experiment PBR@LSR (Photobioreactor at the Life Support Rack) shall demonstrate the technology and performance of a hybrid life support system under real space conditions during an operation of half a year. To be launched to the International Space Station (ISS) in 2018, PBR@LSR combines a microalgae photobioreactor (PBR) and the carbon dioxide (CO_2) concentrator of ESA's Life Support Rack (LSR), also known as the European ACLS. Accommodated in the Destiny module, LSR will absorb and concentrate CO_2 out of the cabin atmosphere. A dedicated interface allows the utilization of highly concentrated surplus CO_2 for cultivation of microalgae in the PBR. The microalgae species *Chlorella vulgaris* uses CO_2 to conduct photosynthesis. Biomass is produced and oxygen (O_2) is released. Besides this technical approach of a hybrid life support system, PBR@LSR also pursues scientific goals: stability and performance of a non-axenic long-term cultivation in the µg-adapted PBR as well as on-ground analyses of returned microalgae samples.

This paper highlights different subsystems of the spaceflight experiment PBR@LSR in the final configuration, especially the algae suspension loop, lighting, gas handling, humidity control, liquid exchange. Within the different subsystems, the selection of critical components is explained. The overall system design is verified with experimental data.

Nomenclature

A	=	absorbance
ACLS	=	Advanced Closed Loop System
ASL	=	algae suspension loop
CCA	=	Carbon Dioxide Concentration Assembly (of the LSR / ACLS)
CO_2	=	carbon dioxide
DLR	=	German Aerospace Center (Deutsches Zentrum für Luft- und Raumfahrt)

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EC = experiment compartment
FEP = fluorinated ethylene propylene
FM = flight model
I = light flux
I/F = interface
IRS = Institute of Space Systems (Institut für Raumfahrtsysteme)
ISS = International Space Station
LiED = Liquid Exchange Device
LSR = Life Support Rack
NASA = National Aeronautics and Space Administration
O_2 = oxygen
OD = optical density
PBR = photobioreactor
PBR@LSR= Photobioreactor at the Life Support Rack
µg = microgravity
µgPBR = microgravity adapted photobioreactor

I. Introduction

Future human space exploration will focus on long-term, deep-space missions including journeys to Moon, Earth-Moon Liberation Points, Near Earth Objects and Mars[1-3]. The return to the Moon is often regarded as an important intermediate step towards a manned mission to Mars as it offers the possibility to test and to demonstrate technologies and to close the gap until a Mars mission[4]. Regarding the requirements of the life support system (LSS) to enable these mission scenarios, significant improvement compared to state-of-the-art technologies is needed. Especially sustainable lunar surface missions and long-term missions to Mars will require a high level of regeneration and closure in order to minimize resupply needs. New technologies like a hybrid LSS (a combination of physico-chemical and biological processes) are needed to regenerate oxygen (O_2) from carbon dioxide (CO_2) and additionally close the carbon loop by in-situ food production. Regarding biological components, the use of microalgae seems highly beneficial, as they are very efficient in terms of light utilization, water demand and essentially higher growth rates compared to plants[5,6]. Current studies initiated by NASA evaluate further benefits of aquatic microalgae systems such as radiation shielding[7] and improved metabolism[8].

Newly developed biological components must be analyzed in terms of efficiency and reliability. Special regard shall be paid to the performance of photosynthesis in space environment, e.g. microgravity (µg) and enhanced radiation. Using the ISS as a testbed to demonstrate and characterize these new technologies under realistic conditions is a crucial step needed in order to qualify and to establish new systems for long-term, deep-space missions. Although the ISS is not a perfect analog for deep space, it is much closer than the environment on the surface of the Earth and will provide invaluable operational experience[9].

To be launched to ISS in November 2018, *Photobioreactor at the Life Support Rack* (PBR@LSR) is a technology demonstration experiment and shall prove the synergetic combination of photobioreactor (PBR) as a biotechnological component and ESA's physico-chemical Life Support Rack (LSR), formerly known as Advanced Closed Loop System (ACLS)[10-15]. The spaceflight experiment shall demonstrate the functionality and feasibility of a hybrid LSS in a real space environment onboard the ISS during a six months operation. Inside the PBR the microalgae *C. vulgaris* is cultivated in a non-axenic manner. The experiment shall also show that the long-term cultivation of *C.vulgaris* in its µg-adapted PBR facility is possible in space.

The PBR@LSR experiment and its development was initiated in 2014 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. A process schematic of LSR with the integrated PBR experiment is shown in Figure 1.

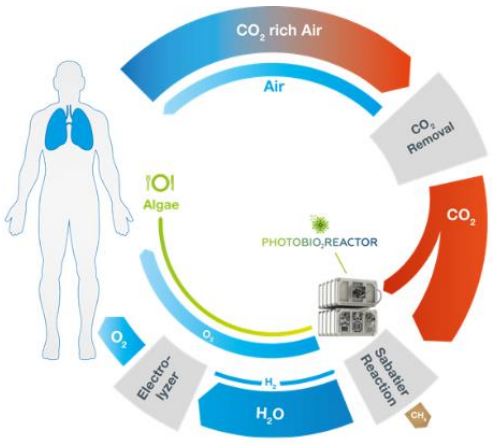

Figure 1: Process schematic of the LSR with the added PBR experiment, a hybrid system approach[13].

Surplus CO_2 from LSR process (or as a backup solution from a buffer) shall be delivered to the PBR without affecting LSR performance. Via the PBR, the CO_2 is converted into O_2. The O_2 will be collected and mixed with residual air in the EC and delivered to the cabin air from time to time. Every two weeks a liquid exchange will allow harvesting of algal biomass and providing fresh nutrients. Several algae suspension samples will be taken at different time intervals and returned to Earth to investigate µg and radiation influence on the algae cells. Several sensors allow evaluating the performance of the experiment.

The main research focuses of PBR@LSR are: the verification of the hybrid system approach, the stability of the algae culture with subsequently a stable gas conversion (CO_2 from LSR into O_2 to cabin air), the production of biomass and operational handling. For the first time, these flight data will reveal the long-term performance, the system stability and reliability as well as the biological stability of a synergistically integrated biotechnological LSS component under space conditions. This paper highlights different subsystems of the spaceflight experiment PBR@LSR in the final configuration, especially the algae suspension loop, lighting, gas handling, humidity control and liquid exchange. Within the different subsystems, the selection of critical components is explained. The overall system design is verified by experimental data.

II. General System Design

The schematic setup of PBR@LSR is shown in Figure 2. Most of the components are located within the gas and water tight Experiment Compartment (EC). Containing a volume of ~10 l, the EC provides access to the Algae Suspension Loop (ASL) and the gas environment.

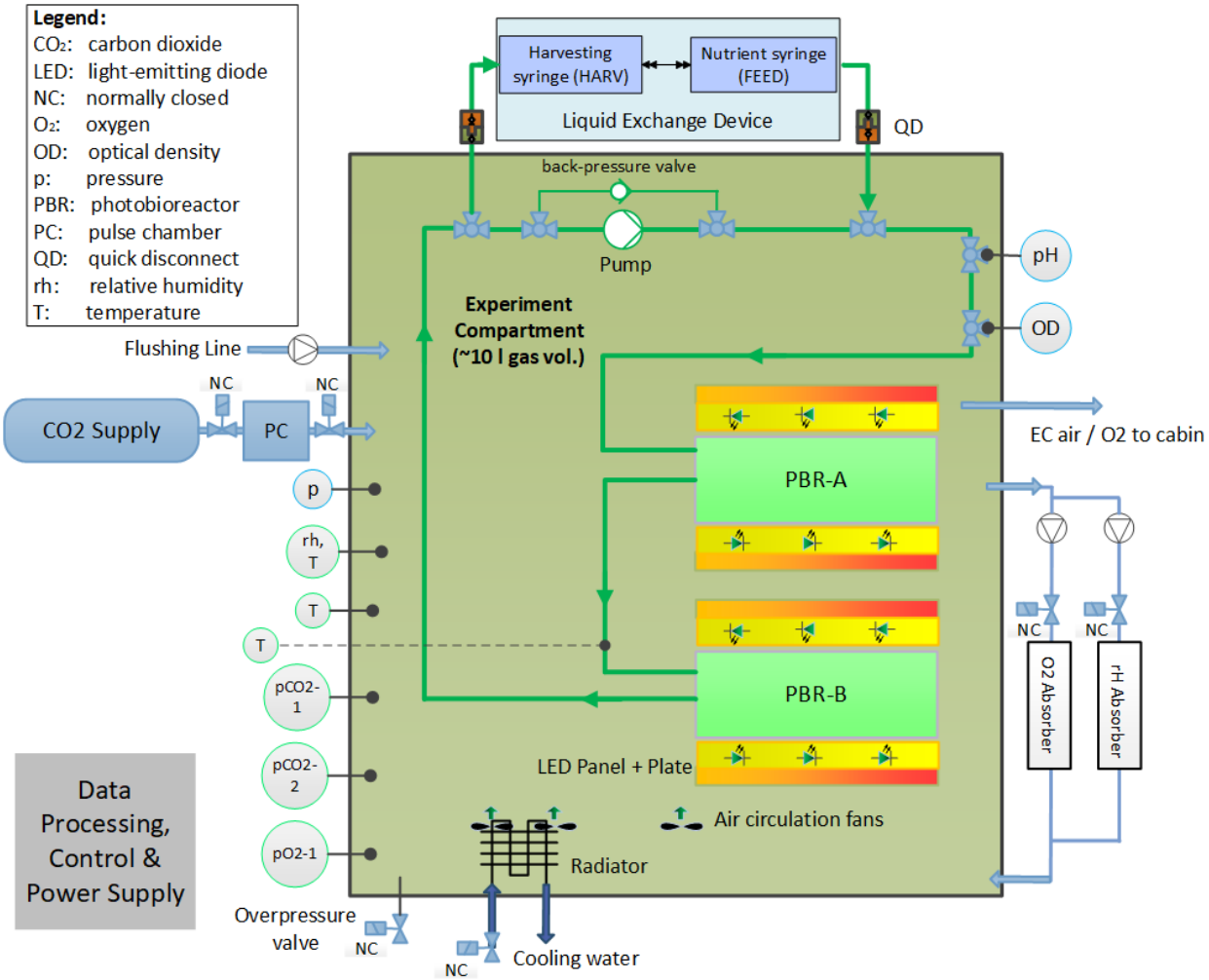

Figure 2: Schematic setup of PBR@LSR.

The ASL contains the algae suspension and is driven by a peristaltic pump. It consists of two µg-adapted photobioreactor chambers (µgPBR) including gas exchange membranes, tubing and connectors, sensors to monitor pH and optical density (OD) and access ports for feeding and harvesting via a separate liquid exchange device (LiED). Custom-built LED panels illuminate the algae suspension and are mounted outside the ASL. A pulse chamber is used to inject CO_2 from the Carbon Dioxide Concentration Assembly (CCA) of LSR into the EC. Air circulation fans constantly mix the atmosphere inside the EC. Several gas sensors are used to monitor the experiment. They include: measurement of pCO_2 (COZIR Wide Range 20%, Pewatron®, UK, sensing principle: nondispersive infrared detector), pO_2 (FIGARO SK25F, Figaro®, Osaka, Japan, principle: sensing galvanic cell), pressure (p), relative humidity (rh) and temperature (T). The temperature is controlled by a cold plate connected to cooling water (16-23 °C) in the Express Rack. Absorbers for O_2 and for relative humidity (rh) are used for atmosphere control.

The connection between PBR and LSR will be realized by a specific interface (I/F) at the LSR, see Figure 4, which allows for routing of excess CO_2 to external experiments. The PBR shall nominally be supplied with CO_2 from LSR. In case of limited access, an internal bottle can provide CO_2 to the PBR. LSR is planned to be launched with HTV-7 and will be accommodated in the US lab "Destiny" in summer 2018[16].

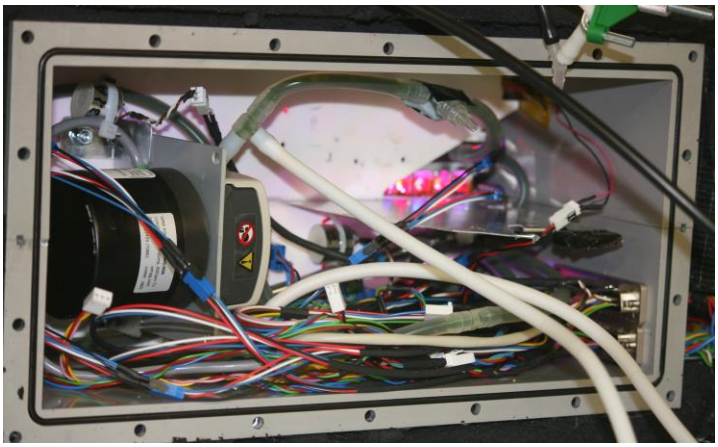

Figure 3: Photography of the breadboard setup of PBR@LSR at the Institute of Space Systems, University of Stuttgart.

Figure 4: PBR CO_2 I/F line.

Figure 5: PBR (left) and LSR (right) = PBR@LSR. A CO_2 buffer is located in the upper middeck locker of PBR.

III. The final configuration of PBR@LSR: Subsystem Design

A. The algae suspension loop

ASL and pump

The ASL contains ~650 ml of culture volume. The peristaltic pump (*Watson Marlow® 114 series*) enables a gentle pumping of the algae cells without introducing too much shear stress[17]. Inside the pump head a high-strength tubing (Gore-Sta Pure PCS®) is used, satisfying lifetime requirements without noticeable wear-out effects. Since any pump introduces mechanical stress to the algae cells by either shear forces, pressure swings or vibrations, the choice of a suitable pump was a crucial decision. The impact of the mechanical stress on the algae cells is dependent

from: the size of the algae, the total number and the frequency of passes through the pump head and the rotating velocity of the pumphead[18]. For this reason the influence of different pump types (e.g. gear pump, membrane pump, peristaltic pump) on *C.vulgaris* was tested at IRS. The selected peristaltic pump performed best and was therefore chosen for PBR@LSR[11].

µg-adapted photobioreactor (µgPBR)

Two µgPBR, found as PBR-A and PBR-B in Figure 2, represent the core of the experiment. They combine the algae culture, lighting, gas transfer and active mixing into one component. Each µgPBR consists of the reactor chamber, containing the culture volume, a gas permeable FEP membrane and the membrane holder with LED panels on top, see Figure 7.

Figure 6: FM of the two µg-adapted PBR.

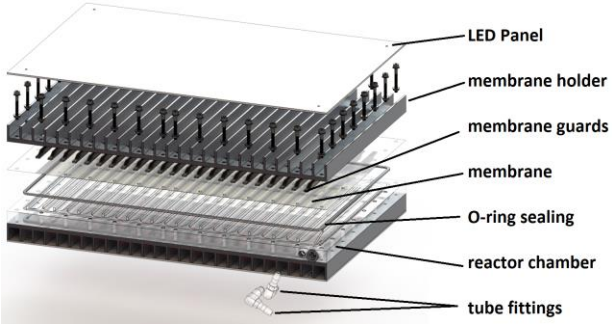

Figure 7: The components of the µgPBR.

The reactor chamber has a meander like flow path allowing a constant mixing of the algae culture resulting in a homogenous suspension and high illumination surface. The homogeneity of the suspension is important for an even distribution of nutrients and algae cells, and also for the execution of regular liquid exchanges with the aim to regulate the biomass concentration inside the PBR system. The reactor chamber is sealed by a gas permeable membrane allowing for sufficient CO_2 and O_2 transfer between the gaseous phase of the EC and the liquid phase inside the reactor. The membrane surfaces are optically transparent allowing a transmission of >98% in the illumination spectrum.

Figure 8: Reactor chamber with meander like flow path.

Sensors within the ASL

Several sensors for the measurement of different process parameters are located within the ASL. They include pH measurement using a flow-through-cell (PreSens FTC-SU-HP5-S, PreSens® Precision Sensing, Regensburg Germany) and an optical density (OD) sensor. The OD sensor allows monitoring the growth evolution of the algae density within the suspension. On ground, the OD is usually measured by photometry of an extracted sample. On board the ISS such a manual action will not be available since crew time is very limited. Thus, an automated OD sensor was developed at IRS specially for PBR@LSR, see Figure 9.

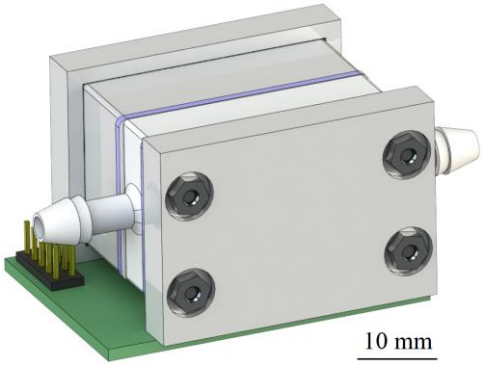

Figure 9: 3D view of the OD sensor.

The OD sensor has a measuring chamber with a gap width of 4 mm that is included within the ASL. Two LEDs and three photosensitive resistors are used for measurement, see Figure 10: one resistor measures the initial light flux I_0 at the LED side of the chamber and the other two measure the light flux, I_1 and I_2 for each LED, after passing through the measurement chamber with the algae suspension inside. Hence, the absorbance A can be calculated by the $\log(I_0/I_1)$ or $\log(I_0/I_2)$. The OD sensor is calibrated before it is placed within the ASL. Therefore algae samples with known OD are used for reference measurement. A correlation between A and OD can then be determined.

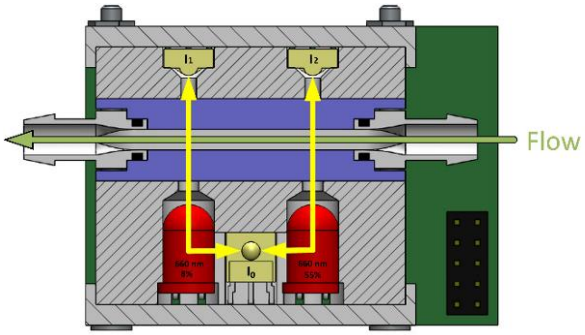

Figure 10: OD sensor working principle.

B. The lighting system

Light impels the photosynthetic process of the algae. The design of the lighting system is crucial since a lacking of light will limit photosynthesis and algal growth. On the other hand, an oversaturation with photons at high light intensities causes photooxidative stress and can damage the photosynthetic system temporary or even permanently by overcharging the pigments[19]. The major chlorophyll components in *C.vulgaris* are *Chlorophyll a* and *b*. This is reflected in the absorption spectrum, see Figure 11. Absorption peaks occur in the blue (435-475 nm) and red (660-680 nm) range of light.

The influence of different light setups for cultivating *C.vulgaris* has been studied extensively[21,22]. Derived from this work, IRS has developed and tested different LED[11]. The experience gained from these developments led to the FM design of the LED panel, see Figure 12. Evenly distributed blue and red LEDs are used for illumination. The red and blue LED strings are controlled separately. Each side of each reactor chamber is illuminated by one LED panel, also see Figure 7. The light intensity of the FM LED panel can be regulated from 0 to 400 µmol/(m²s). The illumination is provided continuously. To protect the electronic parts from corrosion, the LED panels are coated.

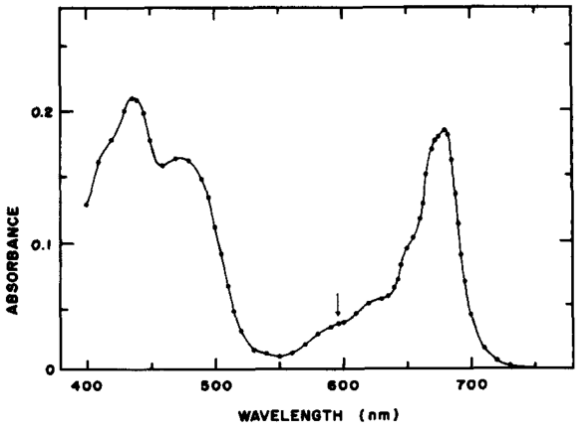

Figure 11: Absorption spectrum of *C. vulgaris*[20].

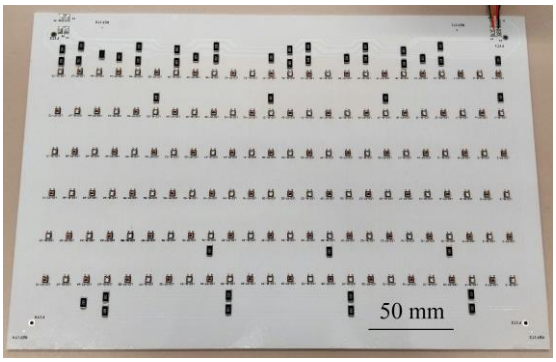

Figure 12: FM of the LED panel.

C Gas handling

To ensure a continuous operation of the PBR system, supplying CO_2 to the microalgae and removing excess O_2 is crucial. During nominal operation, excess CO_2 from LSR shall be supplied to the EC via a pulse chamber. As a backup solution, CO_2 can also be supplied from a buffer. Once the CO_2 is in the EC, it can enter the microalgae culture through the gas permeable FEP membrane. This membrane allows sufficient gas transfer for both CO_2 and O_2. The produced O_2 leaves the microalgae culture through the membrane and accumulates inside the EC. Once the O_2 concentration inside the EC reaches 25%, the EC is flushed with cabin air and the O_2 is released to the cabin.

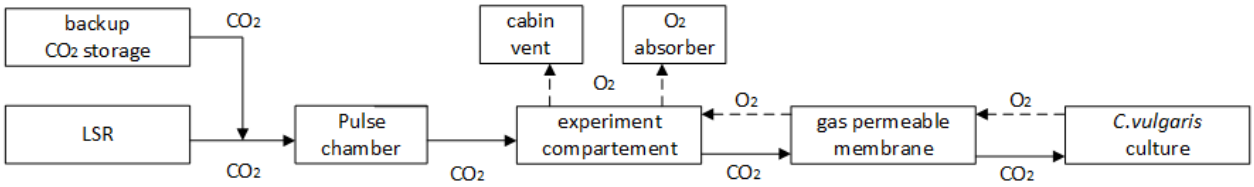

Figure 13: Flow schematic of the gas handling system.

To further decrease the O_2 concentration inside the EC, an absorber is used reducing the O_2 concentration down to 12%. This procedure enlarges the time interval between two O_2 reductions to approximately one week. This helps balancing the O_2 production and CO_2 consumption without disturbing the system too much.

A homogeneous distribution of gas inside the EC is crucial since the gas transfer through the membrane should occur evenly and the gas sensors (two each for O_2 and CO_2) should give signals which are valid for the complete EC atmosphere. Eight cross flow fans (CFR2B, OLC-inc®, Pleasanton, USA) constantly mix the atmosphere inside the EC. Figure 14 shows the gas mixing inside the EC when a) the cross flow fans are off during the insertion of CO_2, b) the cross flow fans are activated and start mixing the gas atmosphere, and c) the cross flow fans are on when the CO_2 is inserted. As can be seen in Figure 14, activated cross flow fans instantly lead to an immediate homogeneous gas distribution.

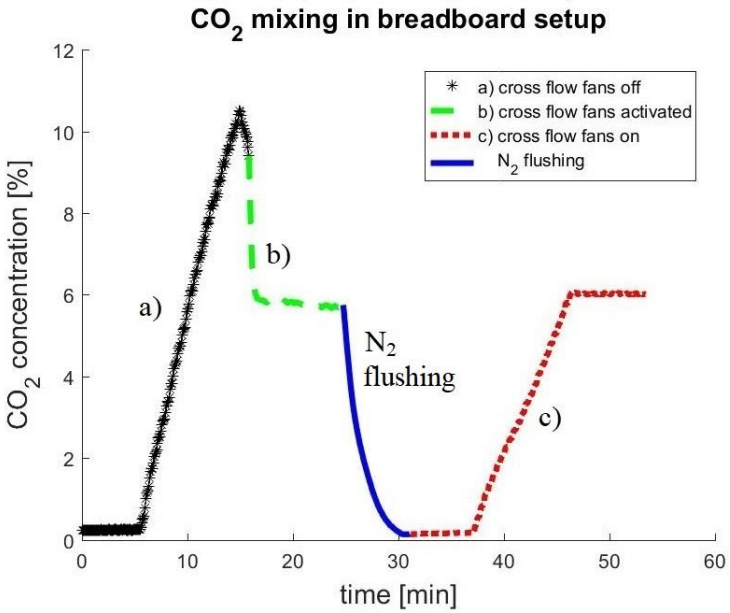

Figure 14: Mixing of CO_2 in the breadboard of PBR@LSR.

C. Humidity control

To avoid the formation of liquid water inside the EC, the relative humidity (rh) is controlled. It is ensured that the rh at nominal operation temperature (27°C ±2°C) is low enough to avoid reaching the dew-point at any region inside the EC. Hence the formation of water drops, especially in the area of the cold plate, which is used for cooling, is avoided. Therefore an rh absorber consisting of silica gel is used. Two rh sensors including temperature measurement, integrated in the CO_2 sensors, constantly monitor the EC atmosphere. A two point regulation is used. When the upper rh limit (60% rh) is reached, a membrane pump is activated, sucking air from the EC through the rh absorber before pumping it back to the EC. The pump is deactivated as soon as the lower rh limit (50% rh) is reached.

D. The liquid exchange device

The operational handling on the µgPBR and the ASL requires the exchange of liquids. This includes the insertion of the culture medium, the inoculation of the microalgae and regular feeding and harvesting. The feeding and harvesting procedure is done every two weeks. Fresh macronutrients are supplied to the microalgae and the biomass density inside the ASL is regulated. Approximately one third of the culture volume is exchanged during this procedure. Therefore, a special Liquid Exchange Device (LiED) was developed. The main requirements to this device[23,24] include the prevention of pressure peaks during liquid exchange (for the membrane of the µgPBR: $\Delta p_{design} < 450$ mbar) and the total volume that has to be exchanged without changing the volume of the ASL. Additionally, leakage must be avoided.

To ensure that no liquids will exit the system, different adapter-interfaces were tested. They have all initially been developed for medical purposes and allow a safe and reliable drip-free connection / disconnection. Liquid can only flow when the adapters are connected. The adapters used for PBR@LSR are B.Braun Safeflow® and CareFusion Texium™.

Syringes are used as reservoirs for the liquids. To save space during transport and storage, the syringes have been modified by making the bolt detachable and reusable. One filled syringe and one empty syringe are inserted into the LiED, see Figure 15. The mechanism of the LiED was designed to ensure, that the liquid input and output occurs simultaneously when the astronaut operates the crank handle. A slip clutch is used to ensure that the maximum pressure is not exceeded, even if one of the syringes would block. The hull around the mechanism of the LiED is designed for maximal safety and most comfort. The cover is transparent to allow the observation of the liquid exchange process (e.g. the moving of the bolts of the syringes). The adapter interface ring in front of the LiED allows the connection to the PBR system. The tubes coming out in front of the LiED are manually connected to the ASL during liquid exchange via the adapter I/F.

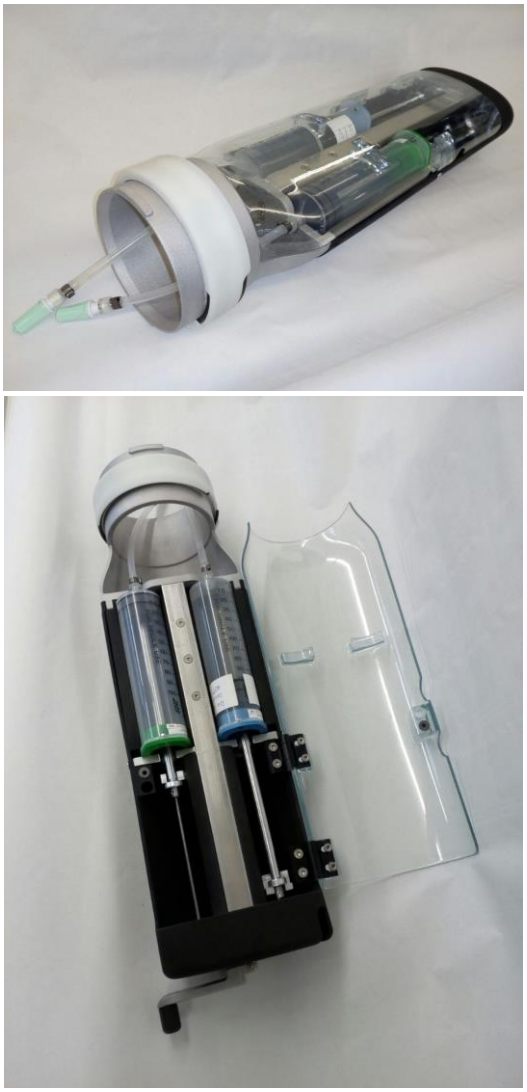

Figure 15: FM of the LiED

IV. Overall system design verification via Ground Testing using Breadboard Setups

Numerous cultivation experiments have been performed during the breadboard phase focusing on the testing of different setting and components, testing the operational handling and mostly on testing the culture stability and performance within this unique setup. Experiment duration varied from few days up to several months. Table 1 summarizes cultivation parameters used during the breadboard phase. In this chapter, the overall system design verification is shown presenting experiment data from a long-term cultivation experiment over 186 days, conducted at IRS in the breadboard setup shown in Figure 3.

Table 1: Cultivation parameters during the breadboard phase of PBR@LSR.

Parameter	Setting
culture volume	~650 ml
temperature	27 (±2) °C
pH	7 (±2)
CO_2 in EC	7-10 %
O_2 in EC	10-25 %
rh in EC	50-80 %
light intensity	100-400 µmol/(m²s)

The microalgae strain *C.vulgaris* (SAG 211-12) was obtained from the Culture Collection of Algae (SAG) at the University of Göttingen, Germany. The culture is non-axenic but free of major contaminants and from other algae strains. The cultivation mode in the breadboard setup is fed-batch and the illumination is continuous.

In the first phase of the experiment, day 0 to day 40, an even distribution of 50% red and 50% blue photons was used for illumination. An initial loss of biomass within the ASL after inoculation (start OD_{680} = 6.3) was observed. Adhesion of the microalgae cells within the reactor chamber and the gas exchange membrane surface might have occurred. This behavior could be explained by an adaption phase needed by the microalgae cells to acclimatize to their new environment and had already been observed in former experiments. A growth of microalgae within the mobile phase of the PBR system was still possible. Between day 2 to day 40, the mean growth rate was 0.03 OD_{680}/day. The lighting regime was changed on day 40 to a molar distribution of 62.5% red photons and 37.5% blue photons resulting into a stepwise release of cell clusters from the reactor chamber and gas exchange membrane, also see picture of microalgae cells on day 82, day 161 and day 178 in Figure 16. The heterogeneous cell distribution within the mobile phase of the PBR system led to a scattering of OD_{680} data. At the end of the experiment, the mean biomass concentration in the mobile phase of the PBR system was OD_{680} = 20. Using the correlation between OD_{680} and dry biomass made for *C.vulgaris* strain SAG 211-12[12], OD_{680} equals to a dry biomass of 4.6 g/l.

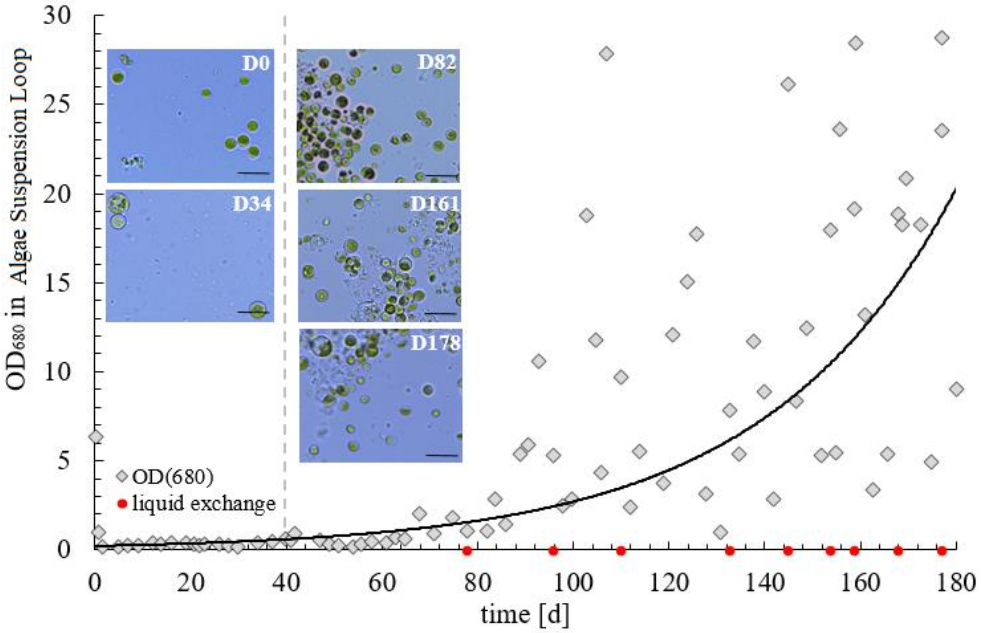

Figure 16: Long-term cultivation of *C.vulgaris* in the breadboard setup at IRS. *The length of the scale shown in the microscopic pictures of the microalgae corresponds to a length of 20 µm. The spline curve relies on a simple exponential function to demonstrate the tendency of algal growth behavior. OD_{680} measurements rely only on cells within the mobile phase, N=1.*

Regular liquid exchanges, harvesting approximately one third of the liquid volume of the ASL and replacing this extracted volume with fresh nutrients, were performed from day 78 onwards, see red dots in Figure 16. A cultivation period of two weeks between two liquid exchanges was chosen for further cultivation runs. During the spaceflight of PBR@LSR a regular crew action will be required by the operating astronaut every two weeks (a total of 13 crew actions during 180 days) with a duration of approximately 15 minutes each.

Figure 17 shows the accumulated gas balance during the long-term cultivation of *C.vulgaris* in the breadboard setup over 186 days. A total amount of 91.7 g CO_2 was consumed (0.493 g/d) and a total amount of 23.4 g O_2 was produced (0.126 g/d). With these values, a photosynthetic quotient (the amount of substance of produced oxygen related to the amount of substance of consumed CO_2) of 0.351 can be calculated.

A steady increase in both, O_2 production and CO_2 consumption was observed until day 40. No metabolic rates were determined between day 40 to day 60. During this phase cultivation problems occurred. Antibiotic treatment was tested without success. After introducing the regular liquid exchanges, harvesting approximately one third of the liquid volume of the ASL and replacing this extracted volume with fresh nutrients, an increase of both, CO_2 consumption and O_2 production was observed again. The gradients of both, CO_2 and O_2 curve were very constant from this time on. The photosynthetic quotient was in a range of 0.3-0.4 during this phase.

The balance study of gas production and gas consumption allows quantifying the performance of the microalgae cultivation in the PBR system. Positive gradients of both consumption and production indicate an operational and working system. The continuity of the gradient of these curves indicates the stability of the system.

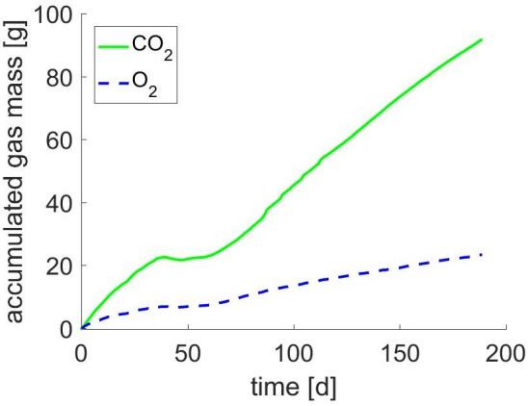

Figure 17: Accumulated gas production and consumption during the long-term cultivation in the breadboard setup.

V. Conclusion and Outlook

To be launched to the International Space Station (ISS) in 2018, PBR@LSR follows the hybrid LSS approach by combining a microalgae PBR and the CO_2 concentrator of ESA's LSR. The development of PBR@LSR was initiated in 2014 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 two µg-adapted PBR, the LiED and the syringes are built by IRS.

This paper presented the final configuration of PBR@LSR and highlighted different subsystems e.g. the algae suspension loop, the lighting system, gas handling, humidity control and the liquid exchange. The functionality of these subsystems was explained and the actual flight hardware was presented. The overall system design verification was demonstrated presenting data from an on-ground long-term cultivation experiment over 186 days in a breadboard setup of PBR@LSR. This experiment demonstrated, that the long-term cultivation in the µg adapted PBR system is feasible. Microalgae growth and gas balancing were evaluated. An interval of two weeks between two liquid exchanges was chosen.

The flight data of PBR@LSR will, for the first time, reveal the long-term performance, the system stability and reliability as well as the biological stability of a synergistically integrated biotechnological LSS component under space conditions. Microalgae samples taken during the experiment will be returned to ground and analyzed. The sequencing of isolated genetic material could be highly beneficial for the evaluation of putative alterations of photosynthesis associated *C.vulgaris* genes, which could have a significant impact on algal photosynthetic performance for permanent application as a biotechnological component in hybrid LSS.

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