Noninvasive process control of a microalgae-based system for automated treatment of polluted agricultural ground water transferred from the development of a biological Life Support Systems

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Microalgae-based photobioreactors (PBR) for Life Support Systems (LSS) are currently being investigated for future space missions such as a crewed base on a celestial body. Biological components can help reducing the required resupply mass by closing material mass flows with the help of regenerative elements. By means of photosynthesis, the microalgae use CO_2, water, light and nutrients to provide oxygen and biomass, which can potentially be used as a food supplement. These capabilities have synergies with Earth applications that address current environmental problems and the already developed technologies can be transferred. For example, a current worldwide discussed issue is the increased nitrate and phosphate pollution of ground water from agricultural waste waters. To investigate the potential use of a biological system - based on the ability of the microalgae to extract and use nitrate and phosphate - for the treatment of polluted ground water, a scalable test stand is being developed, within the PBR@Earth project. This test stand investigates the maximization of intake rates of nitrate and quantifies the produced biomass and oxygen. This paper presents the first steps of the PBR@Earth project, which include the definition of the artificial waste water composition and the current status of the development of the test reactor. At this stage a system concept and a reactor geometry hav been selected. An illumination system has been designed. Future steps in the project, for example the automation strategies and upscaling possibilities are also outlined. Finally, the relevance of such a system and possible applications for the development of a biological component for a LSS is discussed.

Nomenclature

ADP	=	Adenosine Diphosphate
ATP	=	adenosine triphosphate
DSN	=	*Diluted Seawater Nitrogen Medium*
FLE	=	Flashing Light Effect
FPA	=	Flat Panel Airlift Bioreactor
GW	=	Ground Water
IRS	=	Institute of Space Systems
NADPH	=	Nicotinamide adenine dinucleotide phosphate
PBR	=	Photo Bio Reactor
OD	=	Optical Density
SGW	=	Synthetic Ground Water

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I. Introduction

BIOLOGICAL components in Life Support Systems (LSS) are capable of reducing resupply demand by regenerating resources on board a space station, for example regaining oxygen from carbon dioxide and producing edible biomass via photosynthesis and using waste products as nutrients, thus creating possible synergies with other components of the LSS, such as water treatment. These capabilities could have synergies with Earth applications that address current problems and the developed technologies can be transferred. The Institute of Space Systems (IRS) at the University of Stuttgart in Germany, has gained knowledge over the last years on microalgae based components for hybrid life support systems, which are one of the potential biological components for a LSS. The IRS is currently working on transferring the acquired knowledge of process control to terrestrial applications, with the project PBR@Earth, which started in June 2019. The implementation of the technology transfer project was made possible by a grant from DLR Space Management. Project number 50RP1925. One of the possible applications for a technology transfer of a microalgae based system, is the field of water treatment of agricultural waste waters. High levels of nitrate and phosphate in groundwater (GW) can be attributed to the leaching of fertilizers in agriculture and are associated with overfertilisation [1]. This results in the eutrophication of affected waters and is a problem with far reaching effects worldwide, such as the uncontrolled bloom of toxic algae or the poisoning of drinking water [2; 3]. Physicochemical filtration leads to a highly concentrated nutrient solution and shifts the problem to the disposal of said solution [4]. Biological processes offer an alternative [5]. The use of microalgae for the absorption of nitrate and phosphate offers the possibility to add value to the chain of waste water treatment by using the produced biomass as raw material for potentially high value products. Current published data provide values for nitrate absorption by microalgae of up to 200 $\frac{mg}{l \cdot day}$ [6–9]. Additionally, this local CO_2 sink, as well as the nutrient absorption itself, can be offered as a sustainable service option.

The goal of the research project PBR@Earth is the automated treatment of polluted agricultural ground water by using a microalgae-based system. The system shall be able to treat 100 l of contaminated groundwater within one day and use a reactor for the microalgae with a system volume of less than 35 l. For this, a test model will be designed, build and tested in a representative environment. The first steps on the process was the selection of algae species. The microalgae selection process concluded that for this specific application *Chlorella vulgaris* was the most adequate species due to its capability of using nitrate as nitrogen source in various environments such as high ranges of temperatures and pH values. The selection process was described in detail by Martin et al. [10].

This paper presents the next steps in the PBR@Earth Project, the characterization of the ground water and the design of the different subsystems involved.

The goal of characterizing GW is establishing a procedure to create a synthetic ground water (SGW) that represents agriculturally polluted groundwater from Germany. Only German GW is taken into account since the project is based in Germany. Using a SGW is necessary to insure reproducibility. The procedure for the SGW will be verified by testing the produced SGW.

The design of the PBR@Earth test model includes the selection of a reactor, the lighting system, the sensors and the harvesting unit. Main consideration at this stage are the low maintenance, low power, capabilities to determine biomass production and nutrient absorption. A first test-stand, with a reduced volume shall be tested, to evaluate its performance and start the lighting optimization process, before a full scale test-model is built.

Further steps, beyond the scope of this paper are the construction of the full-scale reactor, its automation, further optimization and scaling up to fully fulfill the requirement of nitrate and phosphate reduction of 100 liter of polluted GW per day. These next steps are briefly discussed in this paper as well. Finally, although not part of this project, potential transfer of the gained knowledge to space applications is also outlined.

II. Synthetic Ground Water

To ensure the reproducibility, instead of taking variable natural groundwater, a synthetic groundwater (SGW) shall be defined. Groundwater constituents differ, depending on the nature of the soil and the use of the surface, therefore there is no standardized groundwater available [11]. The selected composition for the here defined SGW is based on the report of the large-scale study on the condition of groundwater in Sachsen-Anhalt as well as legal requirements of Germany for maximum values [12; 13]. The study documented and evaluated groundwater constituents at 1244 measuring points between 2001 and 2010. It was chosen due to its large database and due to the focus of the project on nitrate pollution in Germany. For the composition of the SGW, salts, nutrients and trace substances were considered. Dissolved gases, organic substances, groundwater fauna and drug residues were not taken into account due to the low

concentrations, the high spread and the time dependent decomposition and therefore the reproducibility of the SGW. Heavy metals were also not considered, as their presence in groundwater represents a different type of environmental pollution, which is out of the scope of this project. From 2001 to 2010, at 464 measuring points, approx. 60 measuring points reached the low threshold value according to LAWA (Landesbehörde für Wasserwirtschaft und Wasserrecht) 2004 and 30 of them exceeded the limit value for drinking water according to the Trinkwasserverordnung (TrinkwV) 2011.

For this project, the median of the measured ion concentration in the mentioned report was used to define the main range where 90% of the measurements lie within. The individual ions can be categorized as "cations" and "anions", depending on the charge. In principle, any anion can be combined with any cation to form a salt [14]. The selection of chemicals was based on this previously defined concentration range and aimed to minimize the difference to the median for every single ingredient (Table 1). Exceptions were made for the macronutrients in fertilizers such as nitrate, phosphate, ammonium and nitrite, for which the median and the main range are oriented on the top 10% of the agricultural areas with the highest measured values over the time of the study. Table 1 summarizes the results of the study and presents the calculated concentration of the ions in the SGW. The ingredients, that originated from polluted groundwater, are highlighted in grey. Table 2 presents the selected salts, their solubility in water, the required amount for the composition of the SGW and the resulting concentration of cation and anion from that salt. The resulting concentrations are calculated from the molecular mass and the solved amount [10].

To verify the production process, 5 l of SGW were prepared. During mixing all components dissolved and no observable precipitation occurred. The pH value of the SGW was determined to 6. According to the groundwater report, the pH value should be between 5 and 7. Table 3 shows the results of a laboratory analysis. The ingredients reflect those of the defined SGW. The observed deviations are currently being investigated. Possible explanations could be inaccuracies during mixing, intermediate reactions or measurement inaccuracies.

Table 1. Results of GW study and SGW ingredients

Ingredient		Measured [mg/l]		Ingredient SGW [mg/l]
		Median	Main range	
Chloride	Cl^-	52	11-420	76.
Sulphate	SO_4^{2-}	197	30-550	197
Sodium	Na	22	7 - 80	58
Potassium	K	4.5	1-11	5.2
Calcium	Ca	124	30-230	108
Magnesium	Mg	21	6-50	15
Nitrate	NO_3^-	140	100-367	146
Ammonium	NH_4^+	1.2	0.5-13	2.7
Nitrite	NO_2^-		0.01-0.05	
Phosphate	PO_4	12	1.3-19.9	12.8
Iron	Fe	0.5	0.005-5	0.6
Manganese	Mn	0.09	0.1-0.6	0.4
Aluminum	Al	1	2-14	5.1

Table 2. Selected salts for SGW

Salt	Solubility [g/l]	SGW [mg/l]	Cation [mg/l]	Anion [mg/l]
NaCl	358	10	3.9	6.1
$NaNO_3$	874	200	54	146
$(NH4)2SO_4$	754	10	2.7	7.3
KCl	347	10	5.2	4.8
$CaSO_4$	2.4	340	100	239
$FePO_4$	0.2	1.5	0.56	0.94
$MnCl_2$	723	1	0.44	0.56
$AlCl_3$	450	25	5.1	19.9
$MgCl_2$	1670	60	15.3	44.7
$CaHPO_4$	0.1	17	5	11.9
$Ca(OH)_2$	1.7	5	2.7	2.3

III. Development of test reactor

For the test reactor, first the objectives and the system concept are defined. The main subsystems to be designed and built are the reactor itself, the illumination system and the sensors unit.

A. Objectives

The developed reactor system shall use nitrate and phosphate from polluted ground water as defined in chapter II, to cultivate microalgae and provide a value adding link to the chain of water treatment by offering a purified groundwater and separated algae biomass. Contamination of the GW with algae biomass should not occur. After the filtration, the values of nitrate and

Table 3. Measured ingredients in SGW

Ingredient	Measured [g/l]	Planned [mg/l]	Deviation [%]	Anion [mg/l]
Na	51.1	59.3	16.05	8.20
K	10.1	5.24	-48.12	-4.86
Ca	105	107.8	2.67	2.80
Mg	13	15.3	17.69	2.30
Fe	0.62	0.56	-9.68	-0.06
Mn	0.41	0.43	4.88	0.02
Al	4.85	5.06	4.33	0.21
SO_4	220	197	-10.45	-23
NO_3	177	146	-17.51	-31
PO_4	12	12.82	6.83	0.82
Na	51.1	57.93	13.36	6.83

phosphate shall always be below legal limits. The reactor system shall not be limited to the SGW, but work with different types of GW. To further identify subsystems and components, objectives and requirements are determined. The main objective is to maximize the capability of nitrate uptake and the biomass productivity in relation to the required energy and maintenance efforts. Another objective is the scalability of the system and transferability of cultivation optimizations from a small scale teststand to a larger system. The system shall be able to run automatically without requiring human interference. For the selection of the components, required maintenance time, e.g. for cleaning costs of acquisition and expected lifetimes shall be considered. The cultivation will not occur in an axenic environment, but the selected organism shall be the dominant organism at all times.

B. System concepts

Microalgae are cultivated in PBR, their geometry plays a role in the cultivation performance as it influences gas and mixing rates as well as the illumination distribution in the culture medium [15]. A PBR is a component in a larger system for the task of groundwater treatment. The task of the system is to remove nitrate and phosphate from polluted GW and to upgrade them by means of biological conversion into microalgae [16]. To achieve this, the extraction of nitrate and phosphate of the groundwater can be separated from the biological system and then fed into the PBR as shown in figure. 1. Prefiltration comes with the advantage that the cultivation is separated from the GW. That eliminates the risk of cross contaminating the treated GW with microalgae and allows to control and maintain the cultivation conditions in the PBR for changing concentrations in the GW. The cultivation can be run at the most efficient concentrations, even if these concentrations are above legal limits for GW. Fluctuations in the quantities of substances in the GW will not influence the cultivation. A Physico chemical prefiltration will also increase the buffer capabilities and the modular expandability. For this project, the concept of prefiltration was chosen for further analysis due to decisive advantages:

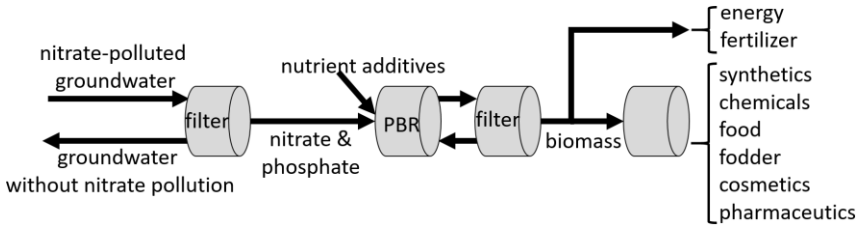

Figure 1. Concept of prefiltration and postfiltration

- Separation of cultivation from GW
  → no risk of GW contamination
- Higher concentrations in the reactor
  → smaller reactors possible
- Dosage of nutrients in the reactor
  → reproducible process control
- Fluctuations in the quantities of substances in the GW have no influence on cultivation
- Same applicability for different groundwater types
- Modular expandability / connectivity of the system to other applications

Nitrate and phosphate should be removed from the groundwater by means of an absorber and then fed into the algae reactor. The chosen absorber is based on the principle of ion exchange with chloride ions, making it important to ensure that the legal threshold value for chloride is not exceeded. This lies at 250 mg/l. In order to show the functionality of the absorber, the 5l SGW were purified as shown in figure 2. both nitrate and phosphate content were determined by chemical analysis before and after filtration. Tab. 4 gives an overview of the measured values. This shows that this form of treatment is capable of meeting the requirements.

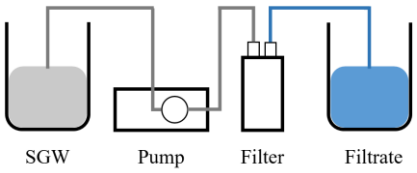

Figure 2. Nitrate & Phosphate absorption test

Table 4. Measured values of nitrate and phosphate

	NO_3^- [mg/l]	PO_4^- [mg/l]
SGW	177	13,6
After Absorption	12	< 5
Threshold	50	10

C. Reactor Geometry

The reactor geometry provides the environment for the microalgae to meet the defined requirements. The geometry of the PBR has an influence on mixing behavior, flow dynamics and light distribution [15]. Another impact of the geometry is on the biofilm formation, the required cleaning effort and manufacturing costs. To increase the energy efficiency of the microalgae cultivation, the photosynthetic efficiency can be improved using the flashing light effect. Thereby algal cells are exposed to a high photosynthetic photon flux density (PPFD) for a short time, followed by a dark period, thus guaranteeing a sufficient integrated photon yield. This effect can be used to maximize the growth rate per light energy either with constant illumination by a strong illumination intensity gradient and geometry induced vortices or by a homogeneous light distribution with a blinking illumination unit.

Figure 3 shows 3 potential reactor geometries. For all compared geometries, an air lift driven mixing is assumed. A tubular reactor in blue, in which the air flows from the bottom to the top of a transparent tube and the light hits the surface perpendicular in each point is compared to a flat panel airlift reactor (FPA). For the tubular reactor, the FLE would be realized by a blinking of the light unit, so a constant light distribution is desired. The FPA could use either a blinking light unit from both sides (orange), where also a constant distribution of the light is desired, or a constant illumination from one side and high intensity gradient combined with geometry induced barrel role swirls in the flow (yellow). To compare the three designs a simulation, based on the principal of the Lambert Beer Law of absorption, eq. 1, was carried out [17; 18]. It calculates the resulting light flux intensity for every level of depth by integrating all incoming rays analytically. For the simulation, a light flux I_0 of 400µmol/s·m² was assumed, an illumination with light of 680 nm wavelength and a biomass DS of 0.2312 kg/m³ *Chlorella vulgaris*. This setup was chosen due to the available data on algae- and wavelength specific absorption coefficients ε_s and due to previously obtained experience with light intensities [19; 20].

$$\log_{10}\left(\frac{I_0}{I}\right) = \varepsilon_s \cdot DS \cdot L \tag{1}$$

Figure 4 shows the results of the simulation. Due to the assumption that all rays are perpendicular to the surface, a focal point occurs for the tubular reactor at the center. The focusing of light towards the center of the tubular reactor leads the compensation of the absorbed intensity and creates a homogeneous light field. FPA illumination from both sides has a similar light distribution as the tubular reactor (Fig. 4). If the realization of the FLE could be made possible by the illumination unit, these concepts would be more suitable for the desired application due to the simplicity of the geometries. Static mixers complicate the production and the cleaning processes. To evaluate the viability of the red and blue options, a study was carried out to determine whether the FLEs can be induced and save energy by using LEDs and what other advantages are associated with controllable lighting. The tubular reactor was preferred for this application since the cleaning of a tube can be done mechanically while in an FPA, mechanical cleaning is difficult. The surface to volume ratio is smaller, for a tubular reactor compared to a FPA, so less problems with biofilm building can be expected [21; 16]. Fig. 5 shows the CAD-models of the 1 liter small scale model, that is used to validate the functionality, the 6 liter test-stand and the planned scaling to a larger system.

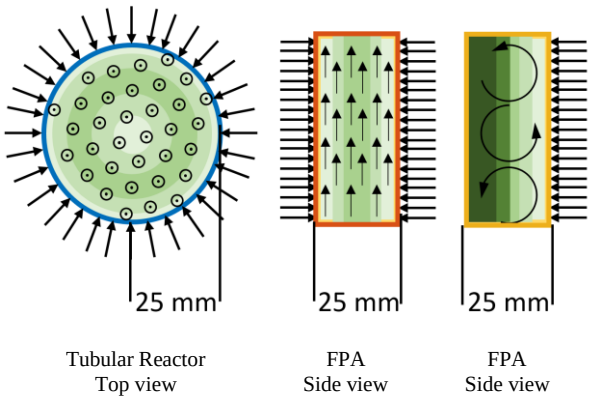

Figure 3. Overview of illumination concepts

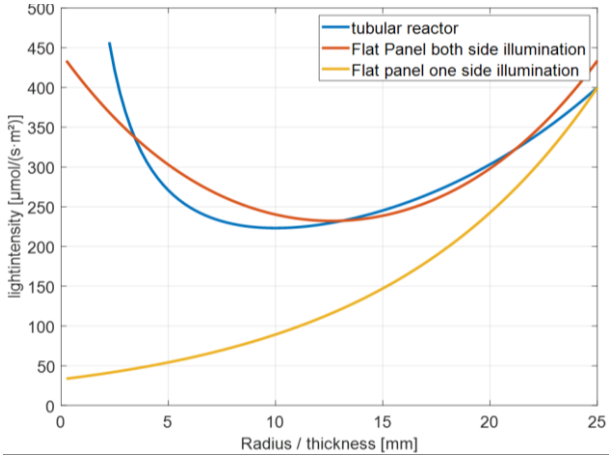

Figure 4. Simulation of light intensities through different reactor illuminations

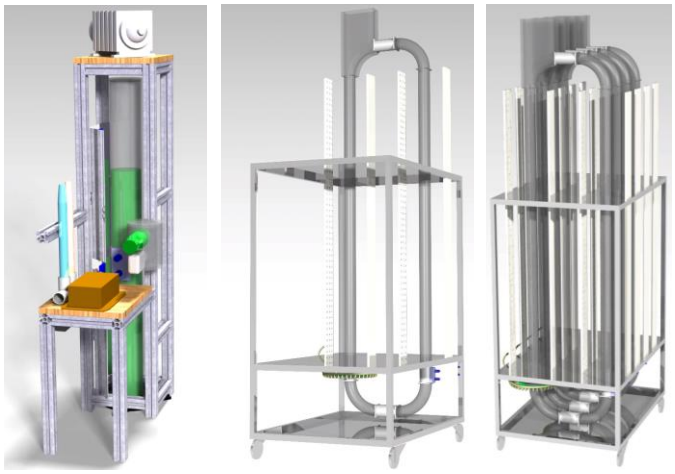

Figure 5. From 1-l model to 6-l test-stand to scaled 24-l-system

D. Illumination system

In general, the illumination unit supplies the algae with sufficient light for photosynthesis. Thereby, the main focus was on minimizing energy input and maximizing energy efficiency, respectively.

Photosynthesis can be divided in two parts, the light and the dark reaction. During the light reaction photons are absorbed by pigments within the plants and used to power an electron transport chain producing chemical energy equivalents. This energy is – independent of light - used for the assimilation of CO_2 and biomass formation in the dark reaction[22]. Technical process optimization for high photosynthetic efficiency is limited and depends on the addressing of bottlenecks in these processes. During the dark reaction it is usually the fixation of CO_2 which is limiting plant growth[22]. An easy approach for optimization is the increase of available CO_2 for the cells. Addressing the light reaction is somehow more complex. Thus, photon absorption depends on the content and composition of certain pigments, mainly chlorophylls and carotenoids (Fig. 6) [23; 24]. As a consequence, there is a maximum amount of photons which can be absorbed and only photons of certain wavelengths within the spectrum are absorbed at all[22; 25].

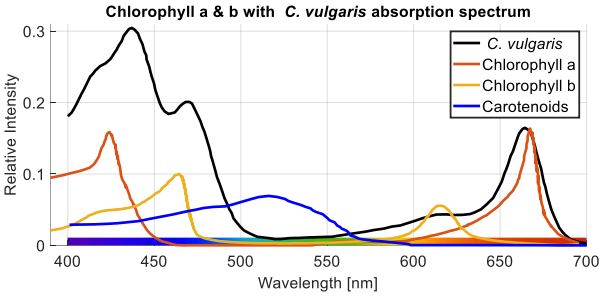

Figure 6. Absorption spectra of different pigments, relevant for photosynthesis, and *C. vulgaris*

The illumination unit developed for this work uses LEDs for high photon efficiency. Different colored LEDs can be adjusted to match the absorption spectrum of *C. vulgaris* or other photosynthetic organisms. Additionally, an adaptable illumination spectrum could also give the option of non-invasive process control [20; 26]

The oxygenic photosynthesis is not capable of using the high amount of photons available in sunlight or intensive synthetic illumination. Excessive energy is lost as warmth or fluorescence. This energy loss can be reduced using the flashing light effect (compare **C).** Furthermore, the risk of harmful light intensities which can appear during constant illumination of a tubular reactor is reduced.

Figure 7 shows the developed light unit from the circuit diagram to the first prototype [25]. The shown light unit has eight types of LEDs with different wavelength, six LEDs of each wavelength are on one module. Modules can be connected to expand the light unit. The light unit is capable of controlling the intensity of each wavelength type individually to control the illumination spectrum, and globally to control the overall intensity. The light unit is also capable of controlling "on – off" periods such as a flashing rhythm to induce the FLE, with periods around one second, and a day-night cycle with periods of 24 h.

At maximum intensity (all LEDs at 100%) of the illumination unit at 10 cm is 1023 µmol/s m², measured with a Kipp & Zonen Par sensor PQS1. Below 200 µmol/s m² at 10 cm distance, some LEDs do not reach the required forward current anymore. Therefore, an operation at lower intensities must be achieved by using dimming screens of increasing the distance to the reactor surface. Figure 8 shows different illumination spectra, normalized on 660 nm compared to the absorption spectrum of *C. vulgaris*. The spectra were produced by the illumination unit and measured with a "Vernier SpectroVis Plus". From an energetic point of view, using solar light as additional light source is beneficial. A problem could occur due to unpredictable fluctuations due to weather effects, heating during daytime and cooling during nighttime. A concept for the use of solar light was developed, that allows the collection of sunlight at times of low intensity, partially shadowing at times of high intensities and thermally insulated closing at times of low temperatures. The sunlight supports the illumination unit, thus reducing the required energy for constant cultivation. By adapting the degree of focus and closure of the mirror unit and the intensity of the artificial light, constant temperatures and levels of illumination can be achieved. Figure 9 shows two possible settings. On the left side, the mirrors function as a collection unit, to increase light intensity in the PBR, on the right side, the unit functions as isolation to the outside and assists the illumination unit by homogenizing the intensity distribution on the reactor surface. However, the development of that illumination system has not been analyzed or developed further within this project.

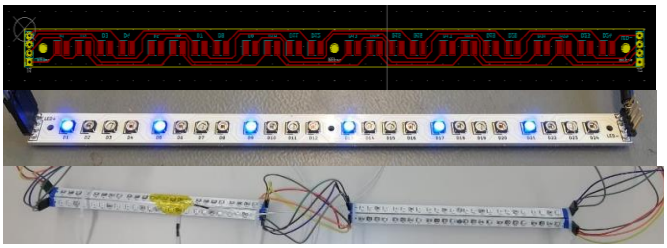

Figure 7. Developed modular illumination unit with eight different wavelengths [20]

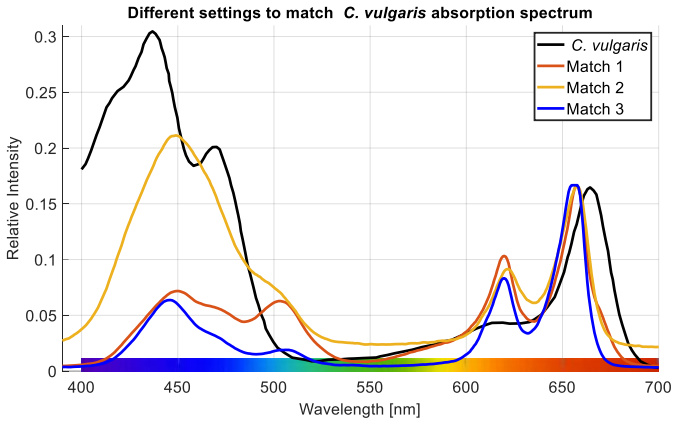

Figure 8. Different spectra settings measured from the illumination unit

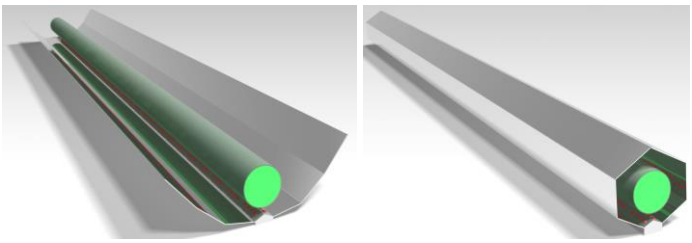

Figure 9. Open and closed setting of sunlight collection unit

E. Sensor unit

In order to optimize the cultivation process with regard to nitrate and phosphate absorption and to implement process automation, various sensors are required in the teststand. The parameters to be measured are temperature, relative humidity, pressure, CO_2 and O_2 in the gas phase and in the liquid phase, temperature, pH, biomass and nitrate ions and solved gasses. Values, within the liquid phase do not change rapidly. Measuring more than three times a day will not increase the accuracy of derived conclusions. By keeping sensors within the algae suspension permanently, a biofilm could form, falsifying measurements. Therefore, a sensor loop was built, as shown in Fig 10 after the reactor operability has been shown. At each measurement point the three-way valves open and enables suspension flow from the PBR into the sensor unit. After measuring, the suspension is pumped back into the PBR and the valves change. Then water from a reservoir is pumped into the sensor unit to flush. After that, the valves change, and the flush water is discarded into a waste-reservoir. Water is pumped into the sensor unit for the time between measurements to avoid drying damages on electrolyte based sensors, such as the pH sensor. This water is discarded before the start of the next measurement cycle. Sensors of the gas phase can be permanently installed in the air exhaust without biofilm formation problems. Tab. 5 gives an overview over the chosen sensors for the develop test stand.

Table 5. Used sensors in sensor unit

Gas phase		Liquid phase	
Measured	Sensor	Measured	Sensor
CO_2	El. Mation SCCO	pH	InPro 3250i/SG/120
O_2	Vernier O2-BTA	Nitrate	Vernier NO3-BTA
T	Allnet ALL3018	conductivity	GDX-CON
rH	Allnet ALL3018	CO_2	InPro5000(i)
P	El. Mation SCCO	O_2	InPro 6000
		T	InPro 3250i/SG/120
		Biomass	Developed

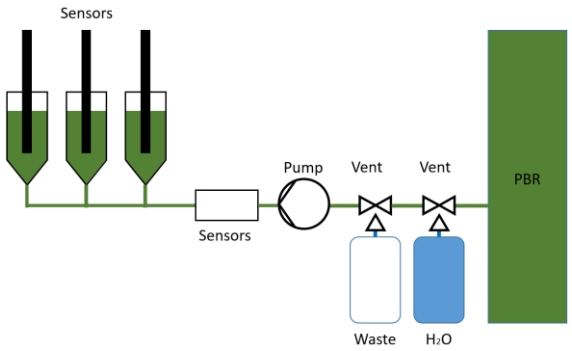

Figure 10. Schematic of sensor loop

IV. Test campaign

The test campaign is divided into two objectives. The first objective of the test campaign was to demonstrate the functionality of the system. To show that *C.vulgaris* can be cultivated in the designed system with nutrients from the defined SGW, a first test was conducted. To compare the achieved nitrate absorption rates in SGW to literature, and evaluate if the SGW has a negative influence on the cultivation, a parallel experiment run was conducted with a Dilluted Seawater Nitrogen medium (DSN). For this first experiment, the settings of Jeanfils [6], concerning gas composition, illumination intensity and nutrient concentration were recreated, to compare the achieved rates to literature. Due to the project requirements, the final reactor should focus on the use of ambient CO_2, yet a high CO_2 concentration environment is used for these first experiments due to the availability of relevant data for comparison. The second objective was, to test the influence of FLE on the energetic efficiency of the PBR. For that another experiment was conducted, where the growth rate and the nitrate absorption is measured in two cultivation runs with same starting conditions – the only difference being the illumination setting.

A. Experiment setup

Fig. 11 shows two one-liter test reactors with adaptable illumination unit that were used to run the experiments. These reactor prototypes were built using 50 mm PMMA-tubes and porous ceramic plates for gas injection. These plates were selected due to the high gas dissolution rates. The used gas was compressed air, filtered through water and a sterile filter, with added CO_2, if required by the experiment. Experiments were run as duplicate in two parallel reactors, with the same algae inoculum to increase the reliability of the measured results. The cultivation cannot be considered axenic. The algae came from the same pre-culture where, in other experiments, a dominance of *C.vulgaris*

of above 95% was measured [20]. For these experiments no such analysis was done. Under a microscope with 600 magnification, no increase of bacteria population could be observed over the experiments. The medium of cultivation was, depending on the experiment either DSN as introduced by Pohl [27], or in nitrate enriched SGW, as defined in section II. The biomass was determined by measuring the absorption at 680nm and calculatin the biomass concentration with a correlation established by Helisch et al. [19]. The nitrate concentration was determined with a chemical reaction and a spectrometer by Hach Lange.

For these first experiments, 1-l reactors were built and used – instead of the larger 6-l reactors in order to conduct more experiments with fewer resources, such as reactor infrastructure, chemicals, and time for cleaning procedures. Later in the project, further tests will be done with the 6-l reactors. It will be evaluated if the measured rates from the 1-l reactors can be reproduced in the 6-l reactors.

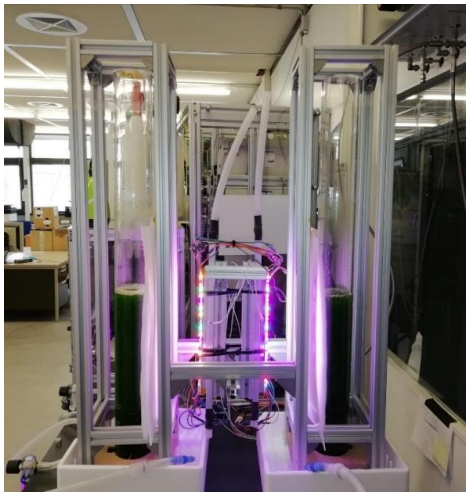

Figure 11. Experiment setup with two 1-l reactors

B. First experiment – Proof of Concept

A first experiment was conducted, to demonstrate the capability of *C. vulgaris* to grow in the chosen reactor type with the developed illumination unit. The illumination was done with 680 µmol/s m² and illuminated from both sides of the reactor. The measured illumination spectrum is shown as "match 2" in Fig. 8. A flashing duty cycle of 0.2 seconds of light and 0.8 seconds of darkness was set. This setting was kept over the whole time - no Day-Night cycle was implemented. This illumination intensity was chosen to provide a comparable maximal illumination as in the experiments by Jeanfils at al [6] to compare the results to literature. The Experiment was conducted with SGW and with DSN. Due to a lower starting biomass concentration in the SGW, the photon flux had to be reduced to 245 µmol/s m² to not photo inhibit the algae. the measured illumination spectrum is shown as "match 3" in Fig. 8. The same flashing rhythm as in the DSN run was kept.

C. First experiment results

Fig. 12 shows the measured results of the cultivation of the first experiments. The orange line shows the nitrate concentration of the cultivation with with 680 µmol/s m² and a 0.2s to 0.8s light to dark setting. The calculated nitrate uptake rate is 97,4 $\frac{mg}{l \cdot day}$ and the achieved biomass production rate, shown in blue, is 155 $\frac{mg}{l \cdot day}$ over 11 days. For the cultivation in SGW, the biomass is shown in grey, and the nitrate concentration in yellow. The growth rates and nitrate uptake rates cannot be compared directly due to the different starting point and illumination scenario, but the experiments proofed that the reactor setup is functional and that nitrate can be absorbed from SGW through microalgae cultivation in this reactor type. These experiments suggest that, for the cultivation in SGW at low light levels, the growth rate per µmol/s m² is higher than for cultivation at high intensities in DSN. With 36% of the photons 73% of the growth rate could be achieved.

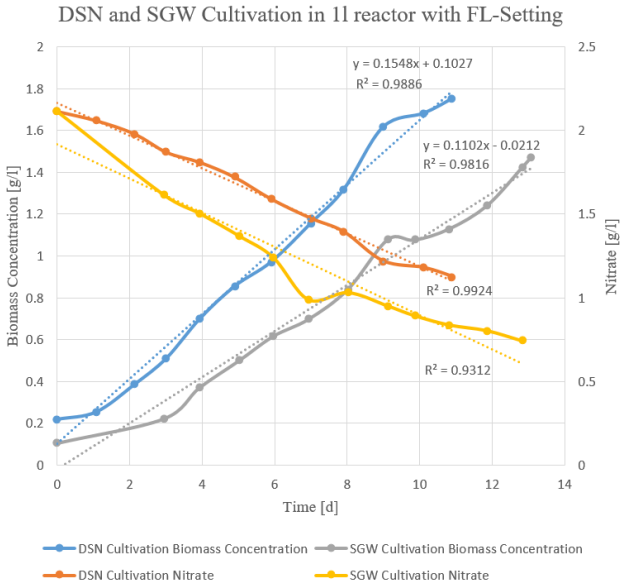

Figure 12. Biomass and nitrate concentration in proof of concept experiment

Jeanfils *et al* used continuous white light at 600 µmol/s m² on a 1-l batch reactor on a shaking table and measured a maximal nitrate uptake rate of 160$\frac{mg}{l \cdot day}$. This can be compared to the measured 97,4 $\frac{mg}{l \cdot day}$. And is within

the same order of magnitude. Other microalgae, in other experimental setups that thus cannot be compared directly also reached nitrate absorption rates in that order of magnitude [7–9]. This indicates that the built system does not have elementary flaws, compared to others and the proof of concept has been presented.

D. Second experiment – Energetic influence of the FLE

In order to quantify the potential to increase the energetic efficiency of the system by using the FLE, two separate cultivation runs were conducted with same starting and operation conditions. The only difference being that one is conducted with constant illumination and the other with the flashing setting enabled. For the first run, the illumination unit was set to constant illumination and to the spectrum "match 1" as shown in Fig. 8. The illumination intensity was reduced to 45 µmol/s m² at the front of the reactor with additional silicone films. This was done to increase the mixing of the single wavelength and avoid peaks. For the second run, the illumination frequency was switched to 0.3s of light period and 0.7s of dark period. The spectrum and the maximal intensity at the reactor surface were kept constant. Both experiments were conducted with DSN medium, a starting biomass concentration of 0.2 g/l and started with a NO_3-concentration of 300 mg/l and a PO_4 of 200 mg/l. The fumigation was 0.5 l/min with 10% CO_2. This setup was chosen to avoid limiting the growth rate via CO_2 limitation. For both cultivations, two runs were conducted to increase the informative value. A further increase in number of parallel cultivations was not possible with the materials at hand.

E. Second experiment results

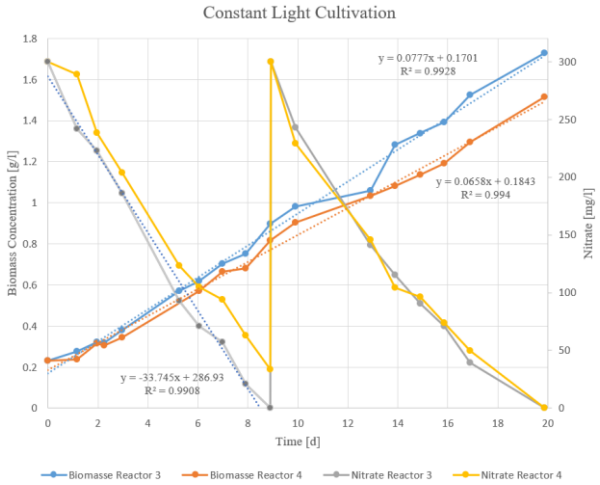

Figure 13. OD_{680} and Nitrate concentration of constant light cultivation with 45 µmol/s m²

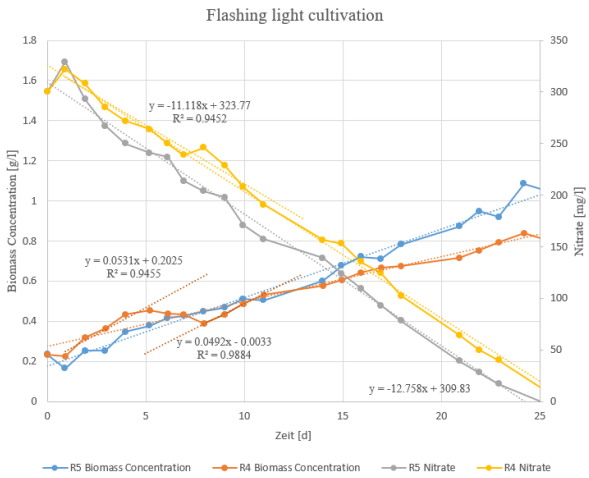

Figure 14. OD_{680} and Nitrate concentration of flashing light cultivation with 45 µmol/s m² and 0.3/0.7s illumination period

Fig 13 and 14 show the measured data of both runs. The shown equations are linear fits to the growth rates. The measured OD at 680nm can be correlated to the biomass dry weight with a linear relation with the factor of 0.23[19]. During the constant light illumination, the nitrate was absorbed in one reactor after 9 days. In order to investigate, whether the original absorption rate will return, both reactors were fed to starting concentration. The reappearance of the original rate suggests that a constant cultivation with this rate could be possible. During the flashing light cultivation, a failure of the CO_2 supply occurred. The failure was discovered and repaired on day 8. Investigations suggest, that the failure occurred between day five and day six. That would explain the decline of the growth rate during that period. Due to the return to the original growth rate after the issue was resolved, this period was ignored for the evaluation of the data.

Tab. 6 summarizes the results of the experiment. The illumination intensity is averaged for the FLE setting and the growth rate related to the intensity of illumination is calculated from this value. The "energetic efficiency" of the

system, related to biomass production more than doubled, and the energetic efficiency of the nitrate uptake rate did not increase that significantly. Since, for this project, energetic optimization of biomass productivity is not a focus, and the increase of roughly 20% in energetic efficiency of nitrate uptake would triple the volume of the reactor, these results indicate that a constant illumination would be preferable. The achieved growth rate related to light energy can be compared to literature, such as Mayo [28] where *C.vulgaris* was cultivated with 80 µmol/s m². The two experiments cannot be directly compared, since the cultivation parameters (such as light, nutrients source, temperature or pH value.) were not exactly the same. However, the system PBR@Earth, after the optimization process that still has not taken place, should be able to achieve comparable values than those already demonstrated in the literature. A growth rate of 0,44 g/l d was reached with comparable temperatures, relating to a energy specific growth rate of 5,5 mg/l d µmol/s m². The nitrate uptake rate related to light energy can be compared to Jeanfils et al [6], here 160 mg/l d was reached with 600 µmol/s m² leading to 0,27 mg/l d µmol/s m². In both cases, the photon efficiency is within the same order of magnitude, however the growth rage achieved differ considerably. This would lead to the need of a larger volume, to absorb the same amount of nitrate within the same time. For the further development, the objective of the project, to absorb the excess nutrients from 100 l SGW within 24 h,

Table 6. Overview over experimentally determined light energy specific rates

Illumination [µmol/s m²]	Growth rate [g/l·d]	NO_3 rate [mg/l·d]	Growth rate rel. to light energy [mg/l·d µmol/s m²]	NO_3 rate rel.to light energy [mg/l·d µmol/s m²]
45	0.072	33.745	1.6	0.75
13.5	0.052	11.93	3.93	0.883

These tests represent the first step in the optimization process. It provides comparable results with the same system set-up both with continuous and FLE. To further optimize energetic efficiency, the effects of different flashing intervals, as well as the intensity of flashes must be investigated. Future experiments shall include cultivations with higher intensities and different flashing settings. Different settings for different biomass concentrations will be analyzed in order to further increase energetic efficiency. Another attempt is to investigate the influence of the illumination spectrum on growth rates and nitrate uptake rates. Up to this point, only the biological degree of efficiency was considered. The degree of efficiency of the illumination unit at different spectra was not measured so far but will be investigated.

V. Next steps

The results shown in the previous chapters have been produced with a test unit of 1 L, working under laboratory conditions. For the further development for Earth applications, both the automation and the scalability will play an important role. Both aspects will also be relevant for space applications, but in that case the boundary conditions for space applications as well as the use of In-situ resources will also need to be considered.

A. Automation Strategy

To further automatize the cultivation process, data from the sensor unit shall be used to adjust parameters. The Biomass-sensor was developed to measure the absorption at four different wavelengths, allowing a correlation to the amount of algal biomass, detection of pigment changes and identification of contaminations with photosynthetic bacteria. By adjusting the spectrum and intensity of the illumination unit, the cultivation process can be influenced noninvasively and the system can react to events. If a certain amount of biomass density is reached, an automated harvest process will be started. If the nitrate or phosphate concentration is below a certain level, a feeding unit will supply the reactor with new nutrients gained from the prefiltration unit of the SGW. Precise numbers of upper and lower limits for biomass and nitrate concentration cannot be given at the moment but will be subject of further investigations.

B. Scalability

Reactor systems that are tested in a lab need to be scaled to relevant sizes. Scaling biological systems could have a negative impact on performance. A 6-l teststand is being built to investigate such effects and validate, if optimizations from the 1-l system can be transferred to a 6-l system. Afterwards, four 6-l systems shall be connected to a 24-l system. This is important, to see if redundancy can be secured and if the reactor system is capable of securing

its functionality, even if one reactor is contaminated. Outside the PBR@Earth project, the 6-l system from the lab could be connected to a 100-l loop, with solar collectors described in III-D. Fig. 15 gives an overview how the scaling to a larger system, could look.

A further improvement from the current lighting system, especially on the larger system, would be the dual use for the lighting system, both as artificial light source and mirror system. Fig 9 the two possible settings. On the left side, the mirrors function as a collection unit, to increase light intensity in the PBR, on the right side, the unit functions as isolation to the outside and assists the illumination unit by homogenizing the intensity distribution on the reactor surface.

Figure 15. Possible scaling form 24-l to100-l

C. Boundary conditions of space applications

After the PBR@Earth project, the developed test-stand can be used to investigate biological components in life support systems on celestial bodies, such as Mars. The 100-l system with mirror collectors isolates the PBR thermally, when closed. If that isolation, plus the heating by the LEDs is enough to secure temperature levels in a desired range (20°C – 30°C) during the nights on Mars, a system like this could be used in a Martian LSS, outside of the habitat. Low outside pressures, sandstorms as well as space radiation also needs to be considered during the system design. In order to reduce the mass that needs to be transported, the use of In Situ resources needs to be evaluated. The largest share of mass in a PBR goes towards the water within the system. Using Martian water as base for cultivation would reduce the start mass. Other possible resources are the CO_2 from the Martian atmosphere, as well as salts, that can be used as nutrients. Using Martian sand to produce reactor tubes would further decrease the dependency from Earth by allowing a scaling of the system without any resupply.

VI. Conclusion and Outlook

The project PBR@Earth shall allow a technology transfer of technologies for the cultivation of microalgae from space applications to Earth water treatment.The current developments have been presented in this paper.

A synthetic groundwater, to represent pollution from agricultural applications and its preparation procedure was defined and verified, variations of 17% for nitrate were measured in an external analysis.

System concepts were evaluated, and key components identified. Different reactor geometry concepts were compared in a simulation of the light distribution and a reactor geometry was selected. The development of the illumination unit and its capabilities of adapting the illumination spectrum and undertake a flashing illumination has been described. Several sensors have been selected for this project and the sensor unit and its working principle have been described.

Two experiments have been conducted. The first experiment was done to verify the functionality of the design. It showed that the system is capable of using nitrate form the defined ground water to cultivate *C.vulgaris*. The achieved growth rates of 97,4 $\frac{mg}{l \cdot day}$ were in the same order of magnitude as comparable literature.

The second experiment was done to investigate the possibility of increasing the energetic efficiency by using the FLE. The optimization experiment indicates that, with the developed illumination unit a significant increase of energetic efficiency can be achieved for the biomass growth rate, comparing constant light illumination with flashing light illumination. In the conducted experiments an increase of more than a factor of two of the energetic efficiency of the biomass productivity was achieved. This increase did not occur in the same order of magnitude for the nitrate uptake rate, here, only an increase of 20% could be observed even thought the absolute uptake rate decreased from

33,74 to 11,93 mg/l d. This would lead to the necessity of a volume increase of the factor 3 to reach comparable total uptake rates. More investigations will follow, with different flashing settings and a focus on ambient CO_2 environments, since the cultivation with higher CO_2 levels, within the PBR@Earth project, would imply a CO_2 concentration unit.

As direct next steps, testing on a 6-l teststand will start. The automation of the sensor unit, and the development of a feed- and harvest unit will take place. Then, the system operating parameters will be optimized to meet the set requirements of metabolizing nutrients from 100 l of SGW in a reactor of 35l within 24h.

In parallel, the gained knowledge could be used to develop a system that can be integrated in a LSS. Further work will be required to analyze boundry conditions and the possibility of ISRU

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