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University of Colorado at Boulder, tobias.niederwieser@colorado.edu

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ANALYSIS OF FACTORS AFFECTING THE IMPLEMENTATION OF AN ALGAL PHOTOBIOREACTOR INTO A SPACECRAFT LIFE SUPPORT SYSTEM

by

Tobias Niederwieser

B.S., Technical University Munich, 2013

M.S., University of Colorado, 2015

A thesis proposal submitted to the

student's graduate committee in partial fulfillment

of the requirement for the degree of

Doctor of Philosophy

Department of Aerospace Engineering Sciences

University of Colorado, Boulder

Fall 2018

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This thesis entitled:

Analysis of Factors Affecting the Implementation of an Algal Photobioreactor into a Spacecraft Life Support System

written by Tobias Niederwieser

has been approved for the Department of Aerospace Engineering Sciences.

____

David Klaus, Ph.D.
Professor, Aerospace Engineering Sciences

____

Patrick Kociolek, Ph.D.
Professor, Ecology and Evolutionary Biology

Date_____________

The final copy of this thesis has been examined by the signatories, and we find that both the content and the form meet acceptable presentation standards of scholarly work in the above-mentioned discipline.

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Abstract

Niederwieser, Tobias (Ph.D., Aerospace Engineering Sciences)

Analysis of Factors Affecting the Implementation of an Algal Photobioreactor into a Spacecraft Life Support System

Thesis directed by Professor David M. Klaus

Algal-based life support systems offer a promising bioregenerative technology for future human space missions by performing the functions of air revitalization, water recycling, and food production. However, despite six decades of active research, no algal-based life support systems have yet been used in a spacecraft. This dissertation analyzes key factors affecting the implementation of an algal photobioreactor into a spacecraft life support system.

A comprehensive set of optimum parameters for growing *Chlorella vulgaris* in a spacecraft was defined to identify research gaps regarding the influence of atmospheric pressure, gravity, contaminants, and radiation as unique cabin environmental factors. From this starting point, the first known comprehensive publication featuring an international and fully historical review of algal spaceflight experiments was completed. Then, using a newly developed and validated flow-through test stand to measure algal metabolism and growth under altered gas compositions and pressures, it was demonstrated that altered total cabin pressure within spacecraft relevant ranges (8.2-14.7 psia) while maintaining normoxic

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conditions did not affect algal growth or metabolism for the conditions evaluated. Additionally, this dissertation features the first known combination of metabolic measurements with metagenomic analysis of non-axenic cultures representative of spacecraft operational environments. Promoting bacterial contamination, together with a variety of green algal taxa, provides novel insight for interpreting results across different algal metabolism studies. The effect of select typical spacecraft chemical contaminants was also assessed. Finally, a first-order feasibility analysis was conducted that established a minimum algal culture volume of 15 liters as being sufficient to support one human in terms of air and water regeneration under ideal performance conditions. This finding was then used to derive the accompanying infrastructure and support requirements that were incorporated into a conceptual design of the system.

The data obtained from this work can be used to support an Equivalent System Mass (ESM) analysis or trade study for spacecraft implementation. Additionally, this thesis serves as a basis for future modelling and experimental verification work needed to increase the Technology Readiness Level (TRL) of algal life support systems that can ultimately help enable sustainable, long-duration human exploration of space.

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1 Introduction

To date, all human spaceflight missions have either been in close proximity to Earth, relatively short in duration, or received periodic resupply cargo delivery. The Environmental Control and Life Support Systems (ECLSS) employed in early short-duration missions relied purely on physicochemical systems using consumables (Daues, 2006). With further long-term missions to Mars and beyond on the horizon, however, this approach will likely no longer be feasible, so a greater degree of closure is needed (Bagdigian et al., 2015; Barta and Henninger, 1994) as noted in NASA's Technology Area (TA) 06 (ESA, 2012; NASA, 2015). For these types of missions, that are likely to extend one year in total duration, partially self-sustaining systems are necessary. The goal of the research field has hence been termed 'closing the loop' of ECLSS referring ultimately to a complete recycling loop within the spacecraft. This contains the conversion of human and cabin waste to consumables such as oxygen, food, propellant and potable water to the extent possible. Specific tasks for converting the metabolically produced carbon dioxide and water into other products such as oxygen are shown in Table 1.

Several technologies that are in place today on board the International Space Station are already aiming towards that goal. An example is the Sabatier reactor that can reduce the carbon dioxide exhaled by the astronauts to oxygen and methane by using hydrogen and thermal/electrical energy. Also currently in operation on the International Space Station is the water processor assembly that is converting wastewater to potable water by using particulate filtration, adsorption, ion exchange,

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catalytic oxidation, and sterilization (Carter et al., 2013). These systems are reducing the resupply mass and volume for the International Space Station, due to their rates of approximately 42 % for oxygen recovery from carbon dioxide and 74 % for water recovery from urine (Junaedi et al., 2011; Tobias et al., 2011). However, these systems are using a notable amount of consumables such as hydrogen or filters and are incapable of providing food and cannot convert 100 % of the waste products.

Table 1: NASA TA 6.1.1 Technology candidates applicable to this theses (NASA, 2015)

TA	Technology Name	Description
6.1.1.1	Carbon Dioxide (CO_2) Removal (Closed-Loop)	Systems that remove metabolically-generated carbon dioxide from the spacecraft atmosphere to safe levels for the crew and deliver the carbon dioxide to onboard processes dedicated to the recovery of oxygen.
6.1.1.2	Carbon Dioxide (CO_2) Reduction	Systems that recover oxygen from carbon dioxide removed from the spacecraft atmosphere through chemical reactions to reduce the carbon dioxide to other products.
6.1.1.6	Oxygen (O_2) Supply	Systems that provide oxygen from stored supplies or onboard generation systems to meet crew metabolic needs and makeup for cabin atmosphere leakage or re-pressurization makeup.
6.1.2.2	Wastewater Processing	Systems that process urine, humidity condensate, hygiene, CO2 reduction product, and other sources of water generated on orbit that cannot be considered potable without some processing for purification.
6.1.4.9	Packaged Food Mass Reduction	Packaged food mass reduction technology development.

Biological Life Support Systems (BLSS) have been studied as the next generation of life support systems as they are the only feasible means of providing complete oxygen and water recovery while also allowing the production of food

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supplementation (Eckart, 1996; Gitelson et al., 2003; Larson and Pranke, 1999). Within this biological domain, algal systems are promising candidates for human life support due to their fully edible biomass, ease of handling, and fast growth rates. The idea of implementing algae as a life support system technology originated already with the beginning of spaceflight in 1957. More recently, new implementation concepts have been proposed (Cohen et al., 2014; Verseux et al., 2015) and related experiments have been conducted (Alexandrov, 2016).

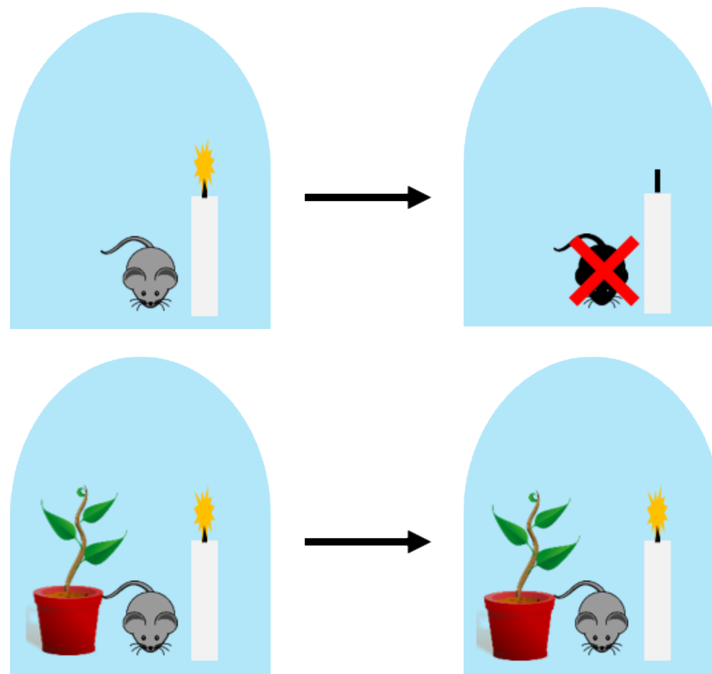

Figure 1: Priestley's experiment leading to the discovery of photosynthesis

In fact, the origins of biological life support systems date back to the 18th century. Most prominently, Priestley in 1771 performed an experiment that ultimately lead to the discovery of photosynthesis, where he enclosed a mint plant and a candle in a sealed bell jar (see Figure 1). Once enclosed, the candle used up all the oxygen and went out, however, after 27 days Priestley was able to relight the

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candle again which proved that plants produce oxygen (Hill, 1971). In 1958, Doney and Myers adapted Priestley's famous experiment and used algae together with a mouse in a closed bell jar instead. It was reported that for the very first time a closed mouse algal system was achieved for 30 days before the oxygen and carbon dioxide levels went out of balance (Doney and Myers, 1958).

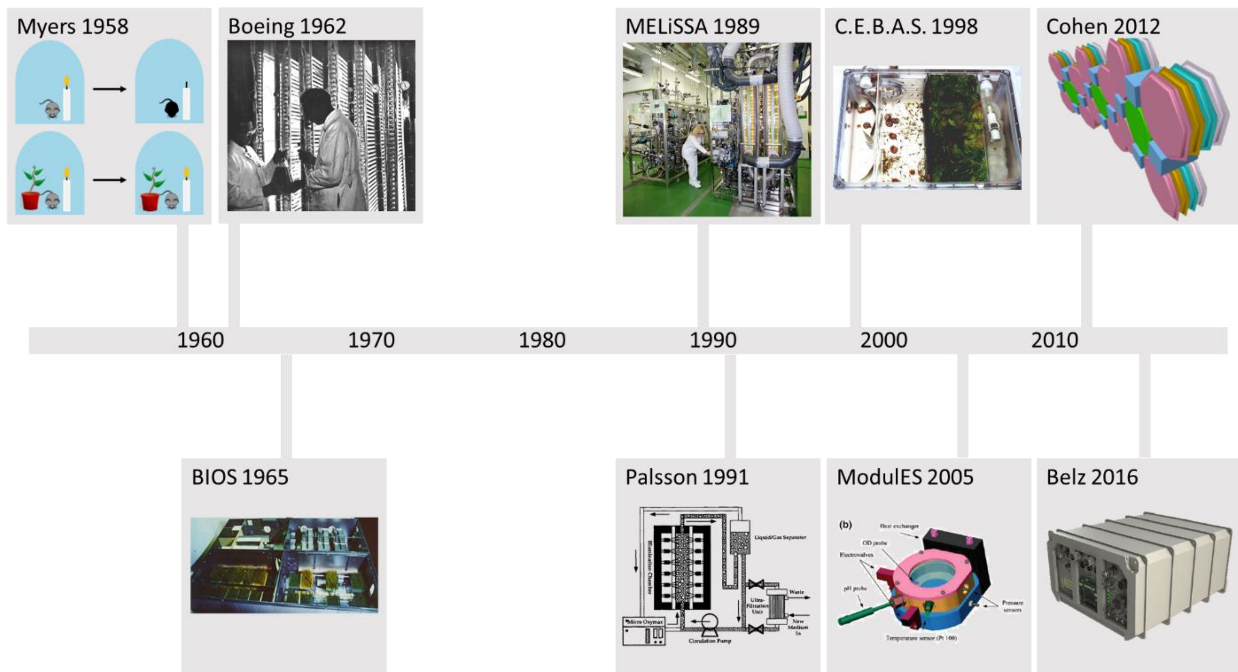

Figure 2: Timeline of algal experiments for spacecraft life support

Due to the success of the early mouse-algae systems, experiments were also conducted with algae and humans soon after both in Russia as well as in the United States as can be seen in Figure 2 (Bovee et al., 1962; Salisbury et al., 1997). One of the problems in these early systems as reported by the leading experts was the power requirement in excess of 4 kW per crewmember at a minimum for providing artificial lighting. State-of-the-art technologies such as fluorescent lights only had an efficiency of 4 % in converting the electrical energy into light (Averner et al., 1984). This high

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power demand together with high thermal loads due to inefficient light bulbs led to the conclusion that algal life support systems are not feasible for spacecraft implementation.

With the advancement of technology and the commercial availability of light-emitting diodes (LED) around 1990, which have efficiencies of more than 50 %, this problem was overcome. It was shown that the growth kinetics as well as the oxygen generation rates in LED lit photobioreactors despite the narrower band of irradiated wavelengths were similar to conventional photobioreactor while consuming less power (Bluem et al., 2000; Bluem and Paris, 2001; Cohen et al., 2012; Javanmardian and Palsson, 1991, 1992; Lasseur et al., 2011; Lee and Palsson, 1994, 1995). As a result, several research groups worldwide have initiated new algal life support systems for spacecraft applications since 1990 and continued research to date (Javanmardian and Palsson, 1991; Keppler et al., 2018).

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2 Background

Algae is a term for a polyphyletic group of aquatic photosynthetic organisms. Algae contain more than 37,000 species most importantly eukaryotic green algae and prokaryotic cyanobacteria as well as many others. It is estimated that more than 50 % of the oxygen we are consuming here on Earth is generated by algae (Kasting and Siefert, 2002). In this dissertation the term "algae" is used mainly for unicellular green microalgae such as *Chlorella* which are edible, fast growing, well described in the literature and tolerant to high levels of carbon dioxide. Macroalgae, more commonly known as seaweed, are multicellular organisms which are not the focus of this dissertation caused by their increased complexity for spacecraft implementation. Algae are living autotroph, or more specifically, phototroph as they are using light energy to reduce carbon dioxide into chemical energy via photosynthesis while usually reproducing asexually by ordinary cell division. The photosynthetic process is occurring in the chloroplasts within the algal cell as shown in Figure 3.

Photosynthesis can be divided in a light-dependent and in a light-independent part. In the former, light energy is absorbed by chlorophyll. In photosystems I and II, water is split into molecular oxygen and hydrogen ions that are binding to $NADP^+$ and are creating Adenosine triphosphate (ATP). In the light-independent reaction, those energy-storing constructs are used to fixate the environmental carbon dioxide to the enzyme Ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO). With the help of NADPH and ATP from the light-dependent cycle, the carbon dioxide is reduced to glucose. The remainder of the carrier enzyme is regenerated to RuBisCO

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using more ATP. This process is called the Calvin Benson Cycle. ATP and NADPH that delivered energy to the Calvin Cycle are transformed to ADP and NADP+ and return to the light-dependent reaction to be reenergized. Outside of the chloroplast, the resulting glucose can be converted into insoluble substances such as oils, fats and starch for storage. For algal growth, glucose and starch can be converted into cellulose that is the basis for cell walls. Additionally, they can form proteins (Raven and Beardall, 2003).

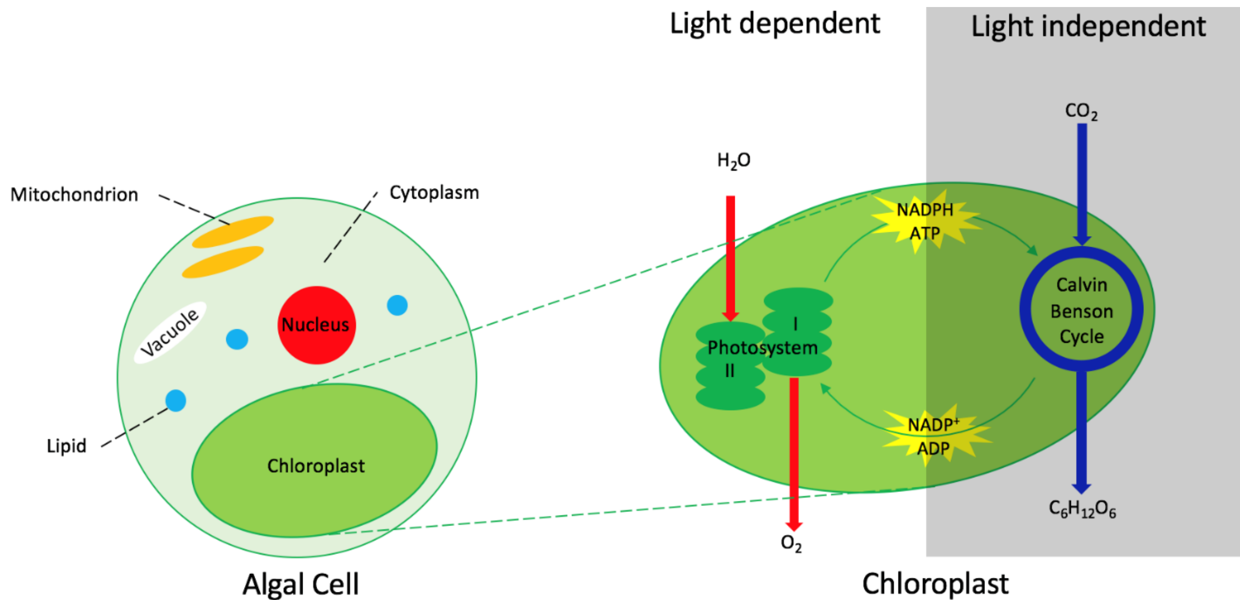

Figure 3: Microalgal carbon dioxide fixation processes

Algae have two methods of taking up the carbon dioxide required for photosynthesis. Firstly, they can take up dissolved carbon dioxide directly from the liquid. Due to the low affinity of RuBisCO to carbon dioxide, algae have carbon dioxide concentrating mechanisms. Gaseous carbon dioxide mechanisms, however, are inefficient as carbon dioxide can easily diffuse out of the cell. Algae have hence developed a second mechanism to concentrate the carbon dioxide using bicarbonate.

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Carbon dioxide dissolved in water can form carbonic acid, which then can lose one or two hydrogen ions to form bicarbonate and carbonate. Bicarbonates can be more easily concentrated than carbon dioxide since they are charged particles. Inside the chloroplast, bicarbonate is then dehydrated to generate carbon dioxide, which can be bonded by RuBisCO. (Moroney and Somanchi, 1999) The hydrogen ions generated in the conversion from carbon dioxide to bicarbonates and carbonates change the pH of the algal solution, which can be used to monitor the starting conditions of the photosynthetic performance (Fondriest Environmental Inc., 2013).

In addition to photosynthesis, which is only carried out during daylight, algae also perform continuous respiration independent of lighting. Here, organic and energy-rich compounds such as glucose react with oxygen from the environment and form carbon dioxide and water. The respiration, however, occurs at a much lower rate than the photosynthesis, causing a net consumption of carbon dioxide and evolution of oxygen during a 24-hour period.

2.1 Cabin environmental effects on the growth and behavior of *Chlorella vulgaris*

2.1.1 Introduction

Chlorella vulgaris Beyerinck is an opportune green algal species for consideration in life support systems, due to its fast growth rates, fully edible biomass (after rupturing the cell wall), high volume efficiency (15 g/l dry biomass production per day), and ease of culturing (Barghbani et al., 2012; Daliry et al., 2017; De Morais

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and Costa, 2007; Lee et al., 2001; Wells et al., 2017). Compared to maximum observed photosynthetic efficiencies (defined as the fraction of light energy converted into chemical energy) of usually lower than 1 % in higher plants, *C. vulgaris* shows light conversion efficiencies of > 3 % (Adamczyk et al., 2016; Melis, 2009). Studies with *Spirulina platensis* (Gomont) Geitler in flat panel photobioreactors even have achieved photosynthetic efficiencies of 10 – 20 %, even though maxima have been theoretically calculated to 6 – 10 % (Janssen et al., 2003; Melis, 2009; Zhu et al., 2008). Most research found in the literature on *C. vulgaris* has been performed for the oil, cosmetic, and food industries. In these industries, maximum biomass production rates, together with high lipid content, are desired for economic reasons and therefore, a variety of research has been published on the optimal environmental conditions for maximum growth of *C. vulgaris* (Alexandrov, 2016; Barghbani et al., 2012; Prakash Rai et al., 2015; Rendon, 2014; Sharma et al., 2012). Due to the unique environment of reduced gravity and launch constraints driven by strict mass and volume limitations, these typical terrestrial processes are not always directly practical for use in a spacecraft. However, given the potential benefits that algae offer in this context, there is considerable interest within the spaceflight community in adapting this terrestrial knowledge to achieve high algal biomass productivity for efficient and regenerative ECLSS functionality (Gonzales, 2009; Verseux et al., 2015; Wagner et al., 2016).

The habitable volume within a human-rated spacecraft is an actively controlled environment that is engineered to maintain acceptable living conditions for the crew.

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Interestingly, the narrow range of operating parameters imposed on the habitat, for example in lighting levels, temperature, humidity, and oxygen concentration, is actually beneficial for predicting performance of biological systems. However, the space environment also poses some uncommon operating conditions such as microgravity and radiation that can affect algal metabolism. Additionally, the small, sealed volume of the habitat, while suitable for humans, results in other undesirable conditions as well; namely, elevated carbon dioxide levels together with increased trace gas contamination buildup, which differs from open cultivation conditions typically found in Earth's atmosphere. Due to the multidisciplinary nature of BLSS design, knowledge of the biological capabilities and constraints together with an understanding of spaceflight cabin engineering parameters is needed to define and conduct a thorough system performance analysis.

This chapter specifically addresses autotrophic culturing of the green algae *Chlorella vulgaris* Beyerinck. There are a variety of other green algae and cyanobacteria that have been researched and applied in terms of suitability for life support systems but are not part of this review. This chapter also does not include genetic engineering efforts, nor does it focus on the differences between the various strains of *C. vulgaris*.

2.1.2 Spaceflight cabin environmental parameters

Historically, a variety of approaches have been utilized to create a habitable atmosphere for humans within the hostile environment of space. Early U.S. spacecraft, including the Mercury, Gemini, and Apollo capsules, had a 5 psia

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(35 kPa), 100 % oxygen atmosphere (Daues, 2006). After the Apollo 1 fire, which was caused by an oxygen-rich and therefore highly flammable cabin environment at 16.7 psia while the vehicle was sitting on the launch pad, a venting protocol was implemented for subsequent flights. To reduce flammability while still maintaining a pressure differential relative to atmospheric conditions on the launch pad, a lower concentration of 60 % oxygen was used at 16.7 psia until the capsule was in orbit when it equilibrated down to 5 psia and 100 % oxygen for the duration of the mission (Chaikin, 2016). The atmospheric conditions were later changed for Skylab to a 72 % oxygen, 28 % nitrogen composition at 5 psia (35 kPa) pressure (Daues, 2006). In 1981, the National Aeronautics and Space Administration (NASA) moved toward using standard sea-level conditions beginning with the Space Shuttle orbiter cabin, and these atmospheric parameters continue in today's ISS Program (Daues, 2006). For pre-breathe operations prior to a spacewalk, however, the orbiter could reduce its total pressure to 70.3 kPa (10.2 psia) with 28 % oxygen to maintain a normoxic (normal oxygen partial pressure of 21 kPa) environment. This was chosen to reduce the risk of decompression sickness of the extravehicular activity (EVA) crewmember as they transitioned to a suit pressure of 29.6 kPa (4.3 psia) and 100 % oxygen (Eckart, 1996). Alternatively, all Russian spacecraft have utilized standard sea-level conditions, beginning with the first manned space mission in 1961 and continuing through the Soyuz variants, as well as for the Mir and ISS space stations (Anderson et al., 2015; Daues, 2006). In summary, there are a variety of acceptable design

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options for atmosphere compositions that all meet the physiological requirements of a crew.

2.1.2.1 Current ISS cabin environmental data

ISS conditions represent the state of the art in space habitats, but this is expected to change for future exploration mission designs as described in chapter 2.1.2.2 below. Within the habitable modules of the ISS, most conditions are generally similar to ones found in sea level laboratories on Earth. This allows direct comparison between ground control-based and spaceflight experiments while having gravity and radiation as the primary independent variables.

As shown in Table 2, ISS cabin air temperature, oxygen partial pressure, nitrogen partial pressure, total pressure, relative humidity, and lighting are all within ranges that are typical for most laboratories on Earth. Aside from reduced gravity and increased radiation, the main difference is with the elevated carbon dioxide concentration as noted above. While standard sea-level conditions of ~21 % oxygen and ~79 % nitrogen are used as set points onboard the ISS, carbon dioxide concentration tends to run on the order of 10x higher than outdoor levels found on Earth, which average around 400 ppm or 0.04 %, due to transport gradients in the removal systems. This undesirable component in breathing air presents medical issues ranging from an elevated incidence of headaches to a potential contributing factor causing vision impairment (James and Macatangay, 2004). Current development is underway to improve the air revitalization system onboard the ISS in order to reduce the carbon dioxide concentration, but as of today, it remains an area

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of concern (Gatens et al., 2015; Mulloth et al., 2010; NASA, 2015). Other factors not typical in terrestrial labs, such as microbial contamination and acoustic noise, are also indicated in Table 2.

Table 2: Current ISS environment (NASA, 2000; Thirsk et al., 2009)

Environmental parameter	**Typical ISS Ranges**
Cabin air temperature	17 – 28 °C avg. 21 – 23 °C
Illumination	108 lux
Carbon dioxide partial pressure	0.1 psia (0.707 kPa)*
Oxygen partial pressure	2.83 – 3.59 psia (19.5 – 24.8 kPa)
Nitrogen partial pressure	10.8 – 11.6 psia (74.5 – 80.0 kPa)
Relative humidity	25 – 75 %
Operating pressure	14.2 – 14.9 psia (97.9 – 102.7 kPa)
Gravity	<1 μg
Radiation	<425 μSv/d
Airborne microbes	< 1000 CFU/m^3
Atmosphere particulate level (ø<0.5 μm)	< 100,000 particles/ft^3
Air velocity	0.051 – 0.203 m/s
Acoustic noise	60 dBA

*Current development is underway to reduce the carbon dioxide partial pressure to 0.038 psia (0.262 kPa) (Gatens et al., 2015)

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2.1.2.2 Proposed exploration atmosphere cabin environment

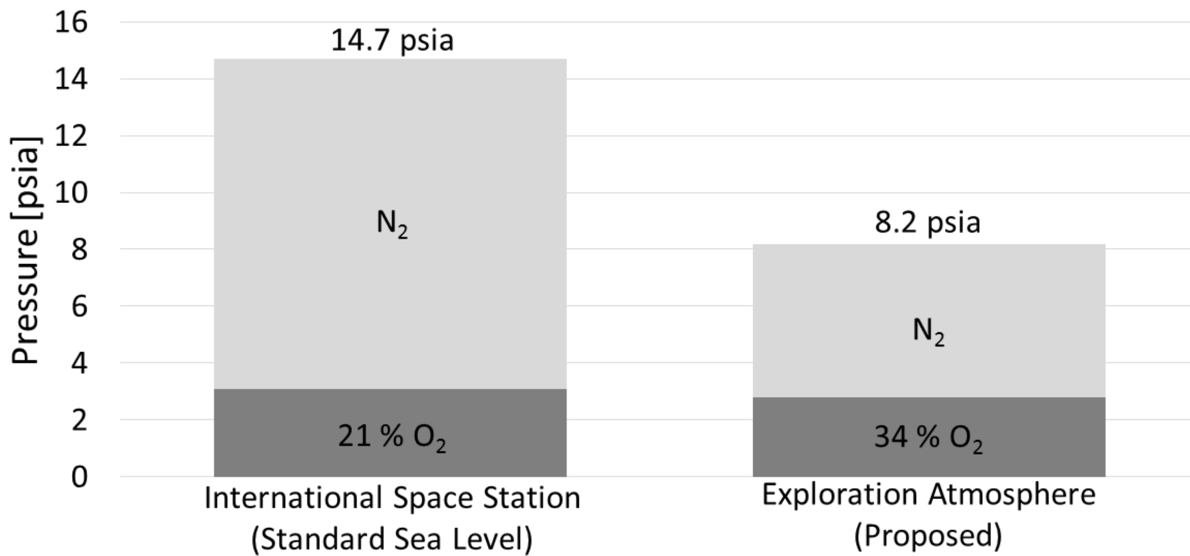

Figure 4: Graphical comparison between current ISS atmosphere (NASA, 2000) *and proposed exploration atmosphere* (Norcross et al., 2013)

To avoid decompression sickness while transitioning from the spacecraft to a spacesuit operating at 4.3 psia (30 kPa), pre-breathe protocols ranging from 4 hours to 2 days using a combination of reduced pressure, increased oxygen and exercise are used to purge excess nitrogen from the blood (Chullen and Westheimer, 2011; Thomas and McMann, 2011). As future long-duration human exploration missions are expected to require spacewalks on a more frequent basis, especially on planetary surfaces, this time-consuming approach becomes impractical. As seen in Figure 4, NASA is therefore proposing an exploration atmosphere of 8.2 psia (56.5 kPa) and 34 % oxygen for future space habitats (Norcross et al., 2013). This combination was derived from an acceptable risk of decompression sickness with minimal pre-breathing efforts on the one side, and flammability limits of materials on the other (Norcross et al., 2013). Another benefit of reduced pressures is the decreased mass

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required for structural containment and minimized leak rates due to smaller pressure differentials between cabin and space, while inducing reduced heat removal and mass transport capacity.

The majority of the remaining environmental parameters, such as illumination or temperature, are expected to remain in comparable ranges. However, as exploration missions move beyond low Earth orbit (LEO) and outside the protective Van Allen belts, the radiation environment for unshielded areas will increase considerably and will also vary with changes in space weather and solar cycles (Tobias and Tood, 1974).

2.1.3 Relevant variables and impact

C. vulgaris cells can grow under a wide range of conditions. For a BLSS, this is beneficial as the green algae offer the potential to develop robust systems that can adapt to different environments (Gitelson et al., 2003). Even so, cultivating algae under ideal conditions is important to optimize volume and mass efficiency. In the following paragraphs, relevant variables are identified and their impact on *C. vulgaris* described.

2.1.3.1 Temperature

The growth rate of *C. vulgaris* increases with rising temperatures from 10 °C until an optimum of 30±2 °C is reached (Barghbani et al., 2012). A further increase to 35 °C slightly decreases the growth rate, and at about 38 °C, the growth quickly drops off, as seen in Figure 5 (Cassidy, 2011; Chinnasamy et al., 2009; Converti et al., 2009; Dauta et al., 1990). This relationship between growth rate and temperature is

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also in alignment with most studies performed with *C. vulgaris* at 30 °C and a calculated theoretical optimum temperature of 32.4 °C modeled in a different study (Mayo, 1997). Batov (1967) has shown that the optimum temperature slightly raises with increasing irradiation levels (Batov, 1967).

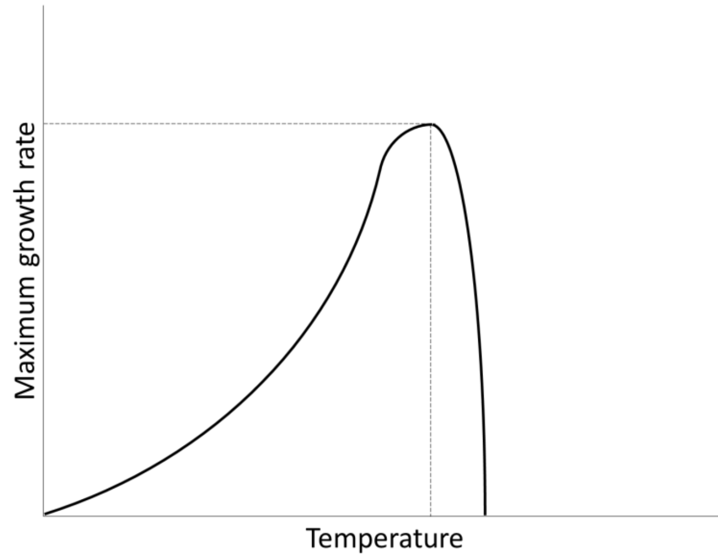

Figure 5: Qualitative relationship between temperature on the maximum growth rate of C. vulgaris (Mayo, 1997)

Since enzymes are involved in both the light-dependent and light-independent parts of photosynthesis, they are primary factors for algal temperature dependence. Enzymes are known to be temperature-dependent in a similar fashion to algae (Daniel and Danson, 2013). Denaturation of enzymes also explains the inhibition of photosynthesis at higher temperatures (Béchet et al., 2013a). However, temperature also alters other mechanisms, such as the fluidity of cell membranes. In this case, the cell is adapting to the reduced cell permeability at low temperatures by increasing the fatty acid accumulation within the cell (Heimburg, 2008). Other observations of cell composition at reduced temperatures show increased amounts of protein and

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starch, and decreased concentration of intracellular free amino acids (Mitsui, 2012; Nakamura and Miyachi, 1982).

2.1.3.2 Lighting

Since light is the main energy source for photosynthesis, algal growth is highly dependent on its characteristics. The three parameters used to characterize the incoming light are spectrum, intensity, and cycle, discussed separately below.

2.1.3.2.1 Light spectrum

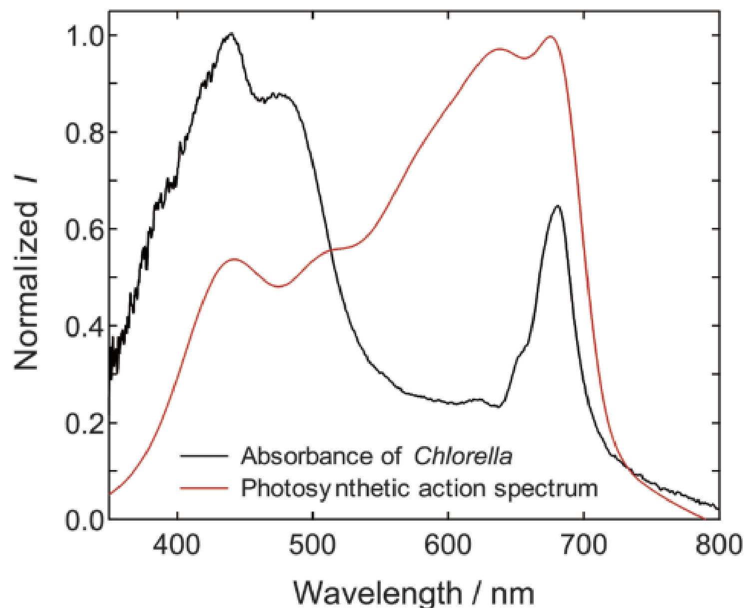

Figure 6: Absorption and photosynthetic action spectrum of C. vulgaris (Nakajima et al., 2015)

Light harvesting pigments within *C. vulgaris* consist of carotenoids and chlorophylls a and b, which absorb the light with wavelengths of 400 – 500 and 650 – 700 nm (see Figure 6), representing blue and red light (Gitelson et al., 2003; Ley and Mauzerall, 1982; Ravelonandro et al., 2008; Yeh et al., 2010). Chlorophyll in photosystems II and I can directly convert light energy at those aforementioned wavelengths to chemical energy. Carotenoids, on the other hand, allow the cell to

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utilize light over a wider spectrum by converting the light to energy and transporting it to the chlorophyll, but at a reduced efficiency. The resulting action spectrum, showing photosynthetic activity at each wavelength, can also be seen in Figure 6. To only account for the light in this active wavelength, the concept of photosynthetically active radiation (PAR) is widespread within the research community (Thimijan and Heins, 1983). With PAR, only the quantitative photon flux in the wavelength between 400 and 700 nm is measured.

In accordance with the PAR light spectrum, Lee and Palsson (1994) have shown that the same growth rates seen under a wide light spectrum can be achieved using light-emitting diodes (LED) with a very narrow spectrum around 680 nm (red) (Lee and Palsson, 1994, 1995). This suggests that algal growth rate is independent from the light spectrum if the spectrum is within the PAR range, which was specifically demonstrated by Kowalik and Schürmann (1984). It was observed that red light irradiation causes an increase in carbohydrate production, whereas a blue irradiation causes an increase in the production of proteins. However, if electrical conversion efficiency is a bigger driving factor than sunlit lighting for the crew, then red and blue light should be provided due to the higher rate of photosynthetic activity in that bandwidth.

2.1.3.2.2 Light intensity

The state of algal cells can be described in 3 different cases: photolimitation, photosaturation, or photoinhibition as shown in Figure 7. Photolimitation starts at complete darkness and is described by a linear correlation between irradiation and

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growth rate. At higher irradiance, photosaturation begins where the growth rate reaches a constant maximum level independent of additional irradiation. This constant level is caused as excess photons are not absorbed in the chlorophyll, but are converted into heat in a process called nonphotochemical quenching, causing a decrease of photon efficiency (García-Camacho et al., 2012). If light intensity is increased even further, algae reach a state of photoinhibition, where an increase in irradiance causes a decrease in growth rate. Any increased light intensity at that point leads to a buildup of oxygen within the cell that ultimately reduces the activity of the oxygen-evolving complex. Under prolonged periods of high light intensity, oxidative damage can kill algal populations (Hakala et al., 2005). It is important to note that the historic unit microeinstein (μE) can be used interchangeably with the more commonly preferred unit of micromole (μmol) with a 1:1 conversion (Vorst et al., 1981). It is beneficial to use the quantitative photon flux ($\mu mol \cdot m^{-2} \cdot s^{-1}$) compared to an energy flux (W/s), as photosynthesis is dominantly dependent on the number of incoming photons. As laid out in chapter 2.1.3.2.1, the energy content of the individual photons, which is equivalent to their spectrum, has little influence on photosynthesis. Bhola et al. (2011) have experimentally established a light curve for *C. vulgaris* and reported good growth at irradiations using a spectrum from 400 to 700 nm between 150 and 350 $\mu mol \cdot m^{-2} \cdot s^{-1}$ with an optimum around 250 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Bhola et al., 2011). This range has been confirmed by other research (Degen et al., 2001; Hassan et al., 2015; Huang et al., 2016). Other investigations have shown

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that the optimum light intensity is independent of light cycles or temperatures (Dauta et al., 1990; Khoeyi et al., 2012; Liao et al., 2014).

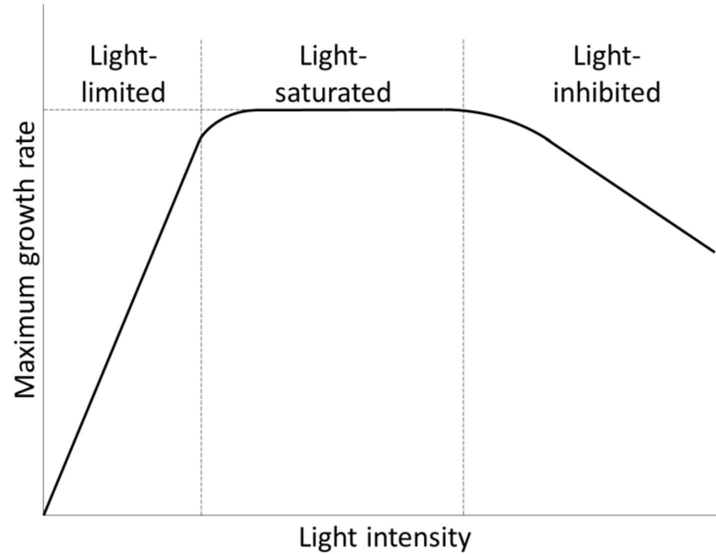

Figure 7: Qualitative relationship between light intensity and the maximum growth rate of C. vulgaris (Béchet et al., 2013b)

2.1.3.2.3 Light cycle

Most of the Earth-based research on *C. vulgaris* assumes the sun as the light source and hence attempts to mimic natural light by using a 24-hour rhythm, and day/night cycles such as 8/16, 12/12, or 16/8 have been commonly employed. Additionally, studies with continuous illumination have also been performed (24/0). It has been shown that longer light periods lead to increased cell growth at different light intensities of 37.5, 65.5, and 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Khoeyi et al., 2012; Seyfabadi et al., 2011). Since the optimum light intensity range of 150 – 350 $\mu mol \cdot m^{-2} \cdot s^{-1}$ was not tested in these studies, this could be an indicator that the culture was generally light-limited. Atta et al. (2013) later confirmed this hypothesis and additionally showed that saturating light intensities and photoperiods above 12/12 yield the highest

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growth rates. Day/night cycles of 16/8, however, have resulted in a 3 % decrease in growth rate. It has also been shown that continuous lighting without any dark period had the lowest growth rate in the light-saturated and light-inhibitive phase (Atta et al., 2013). Species other than *C. vulgaris,* however, have shown a higher growth rate during continuous illumination (Ang, 2004). In the case of *C. vulgaris*, a dark period is needed to produce aplanospores or motile zoospores for reproduction (Krzemińska et al., 2014).

An alternative approach has shown that algae yield a greater growth rate at fast light cycles of several Hertz. This is called the "flashing light effect" (Kok, 1956; Terry, 1986). Rendon (2014) has provided a good overview of research being performed on algae using the flashing light effect (Janssen, 2002; Rendon, 2014). Fu et al. (2012) demonstrated that the flashing light effect is also applicable to *C. vulgaris*. It was further shown that a duty cycle of only 20 % under flashing light results in higher energy conversion efficiencies in the algae and hence increases the biomass yield as well as the growth rate (Fu et al., 2012).

2.1.3.3 Carbon dioxide

Unlike higher plants, aquatic algae do not take up carbon dioxide directly from the gaseous phase but in a dissolved state in the water. When carbon dioxide dissolves in water, it forms carbonic acid (H_2CO_3). By giving off protons, which also alter the pH of the solution, carbonic acid can form bicarbonate (HCO_3^-) or carbonate (CO_3^{2-}), as shown in Equations 1 – 3 (Baba and Shiraiwa, 2007).

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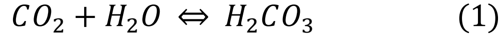
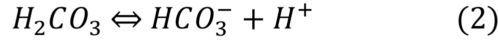
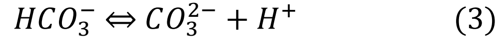


$$CO_2 + H_2O \Leftrightarrow H_2CO_3 \qquad (1)$$



$$H_2CO_3 \Leftrightarrow HCO_3^- + H^+ \qquad (2)$$



$$HCO_3^- \Leftrightarrow CO_3^{2-} + H^+ \qquad (3)$$

As algae take up the carbon dioxide mostly in the form of bicarbonate, it is important to not just control the carbon dioxide concentration but also the acidity of the solution, as outlined in section 2.1.3.4. Since the growth medium also influences the alkalinity of the solution, studies on the optimum carbon dioxide concentration vary greatly. In addition, *C. vulgaris* has been shown to adapt to altered forms of inorganic carbon provisions. Miyachi et al. (1983) have shown that strains of *C. vulgaris* have adapted to uptake only dissolved carbon dioxide, whereas other *C. vulgaris* strains take up bicarbonate and dissolved carbon dioxide (Miyachi et al., 1983).

Studies have exposed microalgae to levels of carbon dioxide between 0 and 100 %. Under pure carbon dioxide atmospheres (100%), they have seen a maximum growth rate at a pressure of 300 mbar. Low survivability at lower pressures is thought to be caused by desiccation, whereas low survivability at higher pressure is due to carbon dioxide toxicity (Thomas et al., 2008). Studies with variable compositions of carbon dioxide in air have found the optimum gaseous carbon dioxide concentration to be between 4 % and 6 % in the headspace of a photobioreactor. It must be noted, however, that the resolution of the tested carbon dioxide concentrations in those experiments is usually higher than 1 %. A 20-fold increase in

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cell concentration in the optimum case over ambient conditions has been reported (Bhola et al., 2011; Chinnasamy et al., 2009; De Morais and Costa, 2007; Singh and Singh, 2014). In addition to the algal carbon dioxide uptake itself, there are two additional factors influencing carbon dioxide uptake – the carbon dioxide transfer rate between the gas and liquid and the ratio of dissolved oxygen, bicarbonate, and carbonate.

To increase the transfer rate between the gaseous and liquid phases, tubular photobioreactors are used to bubble carbon dioxide-enriched air through the algal culture. It was shown that a carbon dioxide concentration of 1 % is sufficient to establish steady gas transfer conditions (Galloway and Krauss, 1961). A recent study, however, has shown that a carbon dioxide concentration of 2.5 % in a sparged photobioreactor is optimal for the growth of *C. vulgaris* (Fu et al., 2012). As sparging is not feasible in a microgravity environment where buoyancy does not occur, breathable membranes have been examined for spaceflight applications. It was shown that this approach does not alter the growth kinetics compared to sparging (Carvalho and Malcata, 2001; Lee and Palsson, 1995). An experimental investigation of the optimum carbon dioxide concentration using a breathable membrane has also shown the optimum carbon dioxide concentration in the gas stream to be 1% (Cheng et al., 2006).

2.1.3.4 pH

It has been experimentally shown that carbon dioxide is partially responsible for changes in pH of the growth medium due to the formation of hydrogen ions, as shown

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in Equations 1 – 3 (Galloway and Krauss, 1961). During algal cultivation, the algae themselves are also responsible for a change in pH due to carbon uptake (Gerardi, 2015). Both low and high acidities can be inhibiting to algal growth, as shown in Figure 8. For *C. vulgaris* the optimum pH has been experimentally determined to be between 7.5 and 8.0 using Bold's Basal Medium (BBM) at a low temperature of 19 °C (Rachlin and Grosso, 1991). However, another study has shown that this optimum range widens if growing the algae closer to their optimum temperature. An optimum growth at 30 °C can therefore be achieved at pH values from 4 to 10 and is a lesser concern for the algal growth (Mayo, 1997).

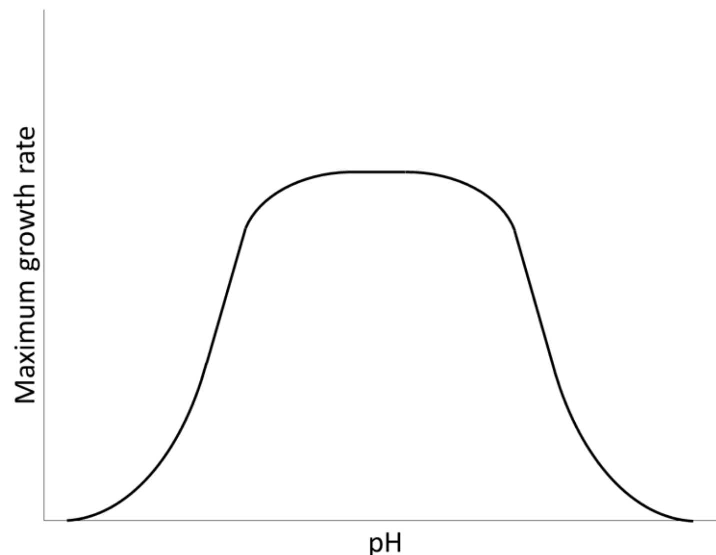

Figure 8: Qualitative relationship between pH on the maximum growth rate of C. vulgaris (Mayo, 1997)

2.1.3.5 Oxygen

Algae are adapted to grow under the normal oxygen concentration of 21 % found in Earth's atmosphere. As seen in Figure 9, increased oxygen gas concentrations can reduce the growth of *C. vulgaris,* whereas reduced oxygen concentrations result in increased growth (Ogawa et al., 1980). This general effect was first described by Otto

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Warburg and is therefore named the Warburg effect (Warburg, 1920). Increased oxygen levels inhibit algal growth by up to 30 % at 65 % oxygen concentration in the headspace (Ogawa et al., 1980). Similarly, carbon dioxide uptake also decreased (by 38 %) with increased oxygen levels at an oxygen concentration of 100 % (Ogawa et al., 1980). The underlying mechanism for this behavior is the affinity of RuBisCO (the first enzyme involved in carbon fixation in photosynthesis) to both carbon dioxide and oxygen (Moroney and Somanchi, 1999). Due to the competition of both substrates, the increase in oxygen concentration inhibits the operation of photosynthesis (Moroney and Somanchi, 1999). Optimum growth conditions for algae may even be achieved in a low oxygen environment, yielding a 14 % increase in growth compared to ambient conditions (Ogawa et al., 1980).

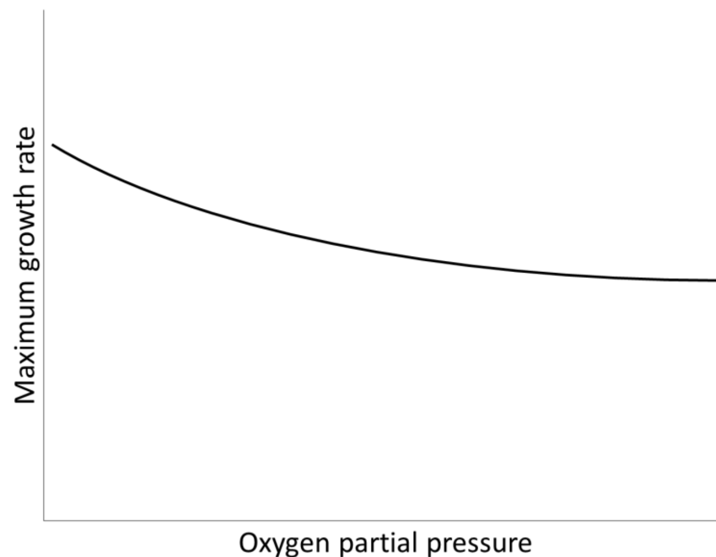

Figure 9: Qualitative relationship between oxygen partial pressure and the maximum growth rate of C. vulgaris (Warburg, 1920)

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2.1.3.6 Pressure

We have not found any reference to studies of *C. vulgaris* using altered environmental pressures. The effects of pressure on other green algae and cyanobacteria, however, have been reported, with results summarized below. Note that in this context, 'pressure' is proportional to the external gas in contact with the liquid, with negligible contribution (< 5 %) from hydrostatic loads.

Orcutt et al. (1970) characterized the growth of green alga *Chlorella sorokiniana* Shihira & R.W. Krauss under a range of hypobaric to hyperbaric pressures (25 Pa – 300 kPa), while holding the carbon dioxide partial pressure constant, in nitrogen balance, and having no oxygen. Interestingly, growth at 25 and 47 kPa was slightly increased, whereas the growth at increased pressures remained unchanged from ambient conditions. Reduced oxygen tension was eliminated as a cause of the increased growth rate with a subsequent experiment, where the diluent gas was exchanged for pure oxygen at the same reduced pressure. In this case, no changes in growth rate were reported. This is a very interesting finding, as high oxygen concentrations at normal pressures result in decreased growth rates. It was also shown that the choice of diluent gas (nitrogen, argon, helium, oxygen and neon) had no effect on growth rate (Orcutt et al., 1970; Warburg, 1920). The physiological processes for this behavior, however, are still unknown (Wagner et al., 2016).

Another experiment performed with cyanobacteria reported reduced growth under a reduced pressure of 50 kPa with fixed concentrations of oxygen and carbon dioxide (Qin et al., 2014a). It was speculated that the reduced total amount of carbon

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dioxide delivered to the cells could have been the reason for this deficiency. The experiment design also required opening the photobioreactor every two days for sampling, which periodically re-exposed the algae to atmospheric gasses and pressure.

Independent experiments, using the EXPOSE-E facility onboard the ISS, exposed desiccated resting stages/colonies of green algae (*Chlorella sp.* ESP-6, *Sphaerocystis sp.* CCCryo 101-99) to space vacuum for about 1.5 years, which were shown to survive in post-flight culturing (Baqué et al., 2017; Cockell et al., 2011).

2.1.3.7 Growth medium

Sharma et al. (2011) tested a variety of growth media with *C. vulgaris* and found that BBM and Chu-10 media resulted in higher growth than Juller's, N-8, or Kuhl's medium. One major advantage of BBM is its widespread use in algal studies. Interestingly, Blair et al. (2014) performed a study that characterized growth of *C. vulgaris* under reduced concentrations of BBM. Their results showed that even higher growth rates can be achieved using only a 50 % concentration of BBM. Further tests have shown that the most probable constituent responsible for the increased growth at reduced growth medium concentrations is phosphorous (Blair et al., 2014). Other media commonly used for microalgal cultivation include Tamiya, Myers, Pratt, and Knop but no direct comparisons with *C. vulgaris* in these growth media formulations have been found.

The interest in *C. vulgaris* for spaceflight applications also originates from the possibility of using human urine and fecal waste water as growth medium. It was

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shown that urban waste waters can achieve growth rates similar to those using BBM (Singh et al., 2017). This ability to reclaim multiple human waste streams (gas, liquid and solid) within a single system employing algae may prove especially advantageous when compared to using separate physical or chemical technologies for each individual process. It was further shown that urine can be used as the sole nitrogen source for an algal culture without any pretreatment (Tuantet et al., 2014a). Additionally, it was experimentally shown that algae are capable of producing drinking quality water as a byproduct of metabolism (Gitelson et al., 2003).

2.1.3.8 Contamination

Most scientific characterization studies with algae are conducted under axenic conditions to eliminate any confounding variables. To maintain axenic cultures a single strain is inoculated in sterile hardware and growth media. In an operational environment with mass cultures, however, it is difficult to maintain axenic conditions. In the small amount of volume in a space habitat used for a multitude of purposes, the potential for cross contamination is high and therefore has to be considered. The type of contamination can be either chemical or biological, as outlined below.

Due to the lack of sedimentation in microgravity, spacecraft cabins can accumulate high concentrations of airborne molecules. Detailed documentation about the contaminants, their acceptable concentration, and crew exposure limits are summarized in NASA's Spacecraft Maximum Allowable Concentrations (SMAC) for Airborne Contaminants document (NASA, 2008). In a manned spacecraft, the

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majority of contaminants listed represents organic matter originating from the crew. *C. vulgaris* has been shown to be tolerant to various organic contaminants found in urban waste water (Singh et al., 2017). This would also be expected in a space habitat, as algae can typically use these as nutrient sources for cell metabolism. Barghbani et al. (2012) identified that high concentrations of Sodium bicarbonate ($NaHCO_3$) inhibit the growth of *C. vulgaris* (Barghbani et al., 2012). As seen with the growth medium, some components, such as ammonia, show a concentration range of optimum algal growth. Very low and very high concentrations of ammonia outside that range both inhibit growth (Tam and Wong, 1996). However, this interaction depends highly on the specific component, as organic solvents like Dimethyl sulfoxide (DMSO) show no inhibitory effect on the growth of *C. vulgaris* whereas ethanol, for example, inhibits algal growth by 95 % at concentrations as low as 1 % (El Jay, 1996).

Hydrogen-based compounds, such as hydrogen chloride, molecular hydrogen, and hydrazine, can also be found in spacecraft at varying levels, and can be toxic to algae. Experiments have been conducted to characterize the effect of herbicides on the green algae *C. vulgaris* (Ma et al., 2002). Another species, *Chlorella sp.* KR-1, has been shown to have high tolerance for NO_x and SO_x fluent gases from power plants (Lee et al., 2000). Barghbani et al. (2012) identified that high concentrations of sodium chloride (NaCl) inhibit the growth of *C. vulgaris*.

Contamination can also occur as microorganisms come into contact with the photobioreactor either through the water or via airborne particles. Non-axenic *C. vulgaris* cultures grown in tap water-based media supported several bacteria species

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belonging to the class of alphaproteobacteria. Surprisingly, this symbiosis with *C. vulgaris* showed only limited effects on overall growth rates and biomass production (Lakaniemi et al., 2012). In fact, it was reported that bacterial contamination (e.g. *Bacillus pumilus*) can even be beneficial for algal growth, with up to 50 % higher *C. vulgaris* cell concentrations achieved (Hernandez et al., 2009).

As future exploration missions are likely to involve operations on the surface of Mars or the Moon, contamination due to Martian or lunar regolith exposure is a further concern. Consideration must be given to the effect of metallic particles found in these environments on algal growth. It is known that a variety of metals can decrease algal growth. For example, Iron (Fe^{2+}), a micronutrient, shows an optimum concentration for algal growth, whereas outside of this optima it acts to inhibit growth (Barghbani et al., 2012). However, it has also been shown that most algal species can recover from metal contamination (Bajguz, 2011).

2.1.3.9 Gravity

Typical Earth-based algal research is conducted under 1g conditions, or in hyper-gravity if a centrifuge is used. In contrast, algal photobioreactors used in space have operated in microgravity. No data on algal behavior was found in the literature pertaining to future partial-gravity, planetary-based systems or in proposed artificial-gravity environments created onboard rotating, orbital space stations. Flight experiments that focused on *C. vulgaris* performance in microgravity conditions are outlined below.

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Algal studies have been conducted in space since the beginning of manned flights in the 1960s. There are two main spaceflight factors thought to influence the growth of algae cultured inside space cabins: radiation and microgravity in addition to hardware-specific influences. The majority of algae experiments conducted in space to date were conducted in LEO, with the exception of the lunar flyby missions, so ionizing radiation exposure is of less concern. Differentiating between the effects of gravity and radiation in flight experiments is difficult, requiring an onboard centrifuge and/or local radiation shielding. However, as most experiments have been of relatively short duration, within the Van Allen belts, and within the spacecraft, the observed effects are likely attributable to the altered gravity levels (Vaulina et al., 1971).

A multitude of algal flight experiments has been conducted in space, but the collection of data from different decades and different locations is challenging and outside the scope of this chapter. It can be concluded that algae were successfully grown in space for a wide variety of different conditions and durations, but a more detailed characterization has not been found. Analysis is difficult as review papers only focus on a specific time, taxa, or space program. In contrast, studies with cyanobacteria are typically generalized in reviews with bacteria as organism (Alexandrov, 2016; Ang, 2004; Dickson, 1991; Harvey and Zakutnyaya, 2011; Kordyum, 1997). A comprehensive review focusing solely on algae flown in space and their photosynthetic properties is still missing. It can be assumed that early hardware limitations influenced the experiments.

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2.1.3.10 Radiation

Ionizing radiation presents a unique concern for long-duration BLSS use, especially outside of the protective Van Allen belts, as it can cause damage to DNA and proteins within the cell (Kovács and Keresztes, 2002). Experiments with other *Chlorella* strains have shown that algal growth is inhibited up to 40 % due to ionizing radiation (neutrons with an energy of 0–800 MeV and dose rate of 0.23 mSv h71 for a total dose of 4.8 mSv) under darkness and under optimum PAR light intensities of 120 and 180 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Rea et al., 2008). At low light intensities of 20 and 70 $\mu mol \cdot m^{-2} \cdot s^{-1}$, however, cells did not show any change in photosynthetic activity. The cause for this result is still unknown. Surprisingly, oxygen evolution rates of algae were unaffected by ionizing radiation and showed the expected increase with increasing PAR radiation as previously described in chapter 2.1.3.2.2. Additionally, it was observed that the damage through ionizing radiation is inversely correlated to the cell diameter (Rea et al., 2008). It was hypothesized that lipids, antioxidants, and enzymes in large cell cross-sections can partially shield internal structures.

2.1.4 Recommendations

Various individual effects of culture conditions on the growth of *C. vulgaris* have been well documented in the literature. Temperature, lighting, air compositions, pH, and growth medium can be designed for optimum growth as shown in Table 3 in comparison to typical spacecraft cabin parameters and are described thereafter.

C. vulgaris grows between 10 and 35 °C and has an optimum temperature range of 29 – 31 °C. This is slightly above the current average operating temperatures in

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spacecraft cabins. Culturing algae in typical cabin temperatures ranging from 20 – 25 °C would be accompanied by a 10 – 20 % reduced growth rate.

Table 3: Summary of optimum growth parameters for C. vulgaris compared to current ISS environmental conditions

Variable	**Optimum range**	**Cabin environment**
Temperature	29 – 31 °C	21-23 °C
Light spectrum	400 – 700 nm	N/A
Light intensity	150 – 350 $\mu mol \cdot m^{-2} \cdot s^{-1}$	108 lux (~1.458 $\mu mol \cdot m^{-2} \cdot s^{-1}$)
Light cycle	12/12 day/night	16/8 day/night
Carbon dioxide partial pressure	Active: 0.15 psia (1.0 kPa) Passive: 0.74 psia (5.1 kPa)	0.10 psia (0.707 kPa)*
pH	4 – 10	Not applicable
Oxygen partial pressure	0 kPa	2.83 – 3.59 psia (19.5 – 24.8 kPa)
Total pressure	Unknown	14.2 – 14.9 psia (97.9 – 102.7 kPa)
Growth media	Bold's Basal Medium	Waste water
Gravity	Unknown	<1 μg
Radiation	Unknown	<425 μSv/d

Lighting in the PAR range is the energy source for photosynthesis and therefore crucial for algal growth. For *C. vulgaris,* this can be provided with either clear white lights or blue and red lights at an intensity between 150 – 350 $\mu mol \cdot m^{-2} \cdot s^{-1}$. As

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current spacecraft ambient light intensity is far below for *C. vulgaris* cultivation, a special lighting system for the algae must be provided. The photobioreactor design is thereby highly dependent on the optical density properties of the culture. Both cell density and size as well as growth medium change the light absorption and have to be accounted for when determining the illumination surface to volume ratio of the reactor (Ugwu et al., 2008). In state-of-the-art reactors, a day/night cycle of 12/12 is optimal, but a 16/8 cycle, which is closer to Earth's conditions, is also acceptable with only minimally reduced efficiencies. Increased growth rates can be achieved using the flashing light effect described above but would likely need to be isolated from the crew residing in the space habitat.

The oxygen partial pressure on the ISS is not optimal for algae growth, but it is what they are naturally adapted to on Earth. The proposed exploration atmosphere has a higher concentration of oxygen which, due to the lower total pressure, ends up being a reduced partial pressure. Compared to current ISS and Earth values, the exploration atmosphere may therefore prove beneficial for algal cultivation in that regard. A further reduction of the oxygen partial pressure is only possible when separating algal photobioreactors from the main cabin, as humans physiologically require a normoxic oxygen concentration. The increased partial pressure of carbon dioxide in spacecraft cabins increases the amount of carbonates provided, thereby increasing algal growth. Further increases are not desirable for human physiological reasons and, in fact, continued technology development is ongoing in an attempt to reduce the current ISS carbon dioxide levels for reasons discussed above. However,

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due to the small closed environment and airflow limitations, Earth atmospheric values of 0.04 % will not likely be attainable. To achieve the highest photosynthetic output, it might therefore be necessary to separately remove carbon dioxide from the cabin atmosphere and subsequently feed the carbon dioxide enriched air to the photobioreactor.

The wide tolerance of *C. vulgaris* for growth relative to pH, from 4 – 10, may be viewed as an opportunity, as it allows passive systems to be employed in reactor design that are not dependent on active pH control. The same is true for using human waste water as growth medium. It has been shown that *C. vulgaris* can achieve similar growth conditions both in urban waste water and in urine as in BBM, opening the opportunity to use the algal photobioreactor for additional functionality in recycling material streams in a spacecraft.

Most of the organic contamination typically observed in spacecraft cabins can be tolerated by an algal photobioreactor in terms of survivability, and in fact, some can even be used as nutrition by the algae. However, performance can be reduced as a function of nutrients, therefore design solutions or operational procedures need to be incorporated for optimal use in a BLSS.

The effect of reduced environmental pressure on *C. vulgaris* is still unknown. Other green algae have been reported to be positively stimulated, but documentation is limited. The specific effects on algal growth under NASA's proposed exploration atmosphere of 8.2 psia with 34% oxygen remain to be determined.

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Altered gravity levels, as experienced during space missions, pose an additional uncertainty for implementing algal-based life support systems. Various research has attempted to characterize the effect of gravity on algal growth, but no conclusive data have been obtained to date. Long-term space radiation exposure can further affect algal physiology, and additional research is necessary to characterize those responses, both at the level of an individual cell as well as for the collective culture.

Using algae within a closed loop system for air revitalization and waste water recycling has already been demonstrated in a variety of experiments (Bovee et al., 1962; Gitelson et al., 2003). Without doubt, the most challenging part in closing the mass loop for spaceflight is in terms of food provision. *Chlorella* has been shown to have a composition consisting of protein, calories, fat, and vitamins, which are a good source of nutrients for humans (Tokuşoglu and Uunal, 2003). Additionally, various health benefits have been reported from the human consumption of *C. vulgaris* as a food supplement (Beheshtipour et al., 2013). A variety of *Chlorella* strains have therefore received the Generally Recognized as Safe (GRAS) certification from the U.S. Food and Drug Administration (FDA, 2017). The cellulosic cell wall of *C. vulgaris* poses a problem for human digestion, but different thermal, mechanical, and biological technologies, some of which are employed on industrial scales, have been developed to overcome this problem (Becker, 2007). Humans require a well-balanced diet, and incorporating algae into food, higher plant, and/or animal streams offers promise to help provide the necessary nutrition mix for long duration space missions,

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but more research is needed on this topic (Beheshtipour et al., 2013; Lee et al., 2008; Radhakrishnan et al., 2015; Tikhomirov et al., 2007).

2.1.5 Summary

In this work, potential spaceflight applications and favorable atmospheric conditions for *C. vulgaris* were identified and compared to current and proposed spacecraft cabin atmospheres. Optimum growth conditions were found to be generally in line with most spaceflight cabin environments, especially in terms of temperature environment, reduced oxygen concentration (under the exploration atmosphere), waste water as growth medium, and pH, where only minor modifications need to be done to current spacecraft designs to accommodate photobioreactors using *C. vulgaris*. The order of magnitude elevated carbon dioxide concentration compared to levels found on Earth actually poses uniquely beneficial conditions for increased algal growth efficiency. Further increases in growth rates due to increased carbon dioxide concentrations and reduced oxygen levels beyond human physiological ranges can increase algal growth, but then require an isolated photobioreactor that cannot directly interact with the spacecraft cabin. Even though *C. vulgaris* cultures appear to be relatively robust to contamination, highly redundant system designs, both in a biological sense, but also in an engineering sense, will be needed due to the potential catastrophic nature of failures associated with life support systems. The ambient cabin lighting system within a spacecraft is inadequate in terms of light intensity needed to support algal growth at efficient rates. A reactor, therefore must provide a designated light source, which can also potentially be used for ambient cabin lighting.

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Further research is needed to characterize the effects of altered gravity, radiation, and atmospheric pressure. Current data on those parameters do not indicate reduced performance and in fact, in terms of pressure, actually indicate increased growth rates. Overall, the results from this review show that algae are a promising candidate for use in a BLSS for future space exploration missions. The potential multifunctionality of a single algal life support system consisting of air revitalization, water recycling, food production, and radiation shielding may prove especially advantageous when compared to using separate physical or chemical technologies for each individual function.

2.2 Resultant publications and presentations

Journal Papers

1. **T. Niederwieser**, P. Kociolek and D. Klaus, "Spacecraft cabin environment effects on the growth and behavior of *Chlorella vulgaris* for life support applications", Life Sciences in Space Research, 16, pp. 8-17, 2018

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3 Problem statement

It is shown that algal photobioreactors are a promising technology for regenerative life support technologies in future human spaceflight missions even though to date no algal-based life support system was flown in space. However, in order to systematically assess the feasibility of an algal based life support system, a chain of research is needed since altered gravity, radiation, atmospheric pressure, and contamination have been identified as potential influencing environmental parameters that need further characterization. Characterization of those spaceflight unique parameters require different approaches ranging from literature review to the development and verification of suitable testbeds. From those biological responses, design recommendations have to be derived to allow a mass-, volume-, and power-efficient implementation of an algal photobioreactor into a spacecraft life support system. Based upon developed design recommendations, an implementation concept can finally be established that will allow the targeted first-order feasibility analysis by comparing a scaled version to state-of-the-art systems. A summary of thesis objectives is presented in Table 6.

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3.1 Synopsis of thesis objectives

In order to answer the problem statement, a systematic approach has been laid out that resulted in the defined objectives 1-6 below. First, an attempt is made to answer the effect of microgravity on algal cultures based on the variety of algal flight experiments conducted in space. In contrast, the effect of total atmospheric pressure on the algal behavior is investigated experimentally by developing a suitable test photobioreactor and subsequently conducting controlled experiments. System robustness is not only characterized from a biological contamination standpoint in the experiments, but also from a chemical contamination view. An assessment of the effect of radiation on algal cultures has been specifically excluded from the scope of this dissertation. The obtained results are then used to conduct a first-order feasibility study to implement an algal photobioreactor into a spacecraft.

3.1.1 Objective 1 – Assess the influence of microgravity on algal growth based on past flight-experiments.

Based on published data from the literature, a comprehensive list of all algal experiments in space is needed to get a complete picture of microgravity research on algal cells. This chapter attempts to review past algal experiments flown in space worldwide from the beginning of spaceflight until today. Experimental methods and results from 51 investigations utilizing either green algae (Chlorophyta), cyanobacteria (Cyanophyta), or Euglenophyta are analyzed and categorized by a variety of parameters, including size, species, and growth duration. The collected data are summarized in a matrix that allows easy comparison between the

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experiments and provides important information for future life support system requirement definition and design. Similarities between experiment results are emphasized and key results out of almost six decades of algal research are documented to assess the effects of microgravity on algal cultures. Common problems and shortcomings are summarized and analyzed in terms of potential solutions. Finally, key research gaps, which must be closed before developing a functional life support system, are identified.

3.1.2 Objective 2 – Development of a novel test bed for flow-through measurements of algal metabolism under altered pressure

To measure the oxygen evolution and carbon dioxide fixation in *Chlorella vulgaris* under altered atmospheric pressures while maintaining constant environmental conditions requires the development of a new test setup. Historically, most experiments have either been open to an ambient environment or conducted in closed systems. For this application, open systems are not feasible, as the objective is to alter the atmosphere. Hermetically sealed closed systems would be possible but are unfavorable to characterize the algal growth under defined conditions as parameters change over the course of an experiment. Hence, a flow-through photobioreactor that is capable of maintaining specified growth conditions for *Chlorella vulgaris* and controlling the pressure in the reactor between 56.5 and 101.3 kPa (8.2 and 14.7 psia) has to be developed. The small-scale reactor is sized for obtaining accurate oxygen and carbon dioxide measurements. Additionally, challenges, such as leak rates, measurement resolution and accuracy, as well as

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water temperature alternating the solubility of carbon dioxide and oxygen, are identified and resolved. The testbed is experimentally validated by leak tests, flow tests, preliminary metabolic measurements, and sampling.

3.1.3 Objective 3 – Effect of varying total pressure on population growth and metabolism of *Chlorella vulgaris* at constant oxygen and carbon dioxide partial pressures

As previously described, the biological effect of altered atmospheric pressure and altered oxygen concentrations on algal cultures is unknown. The novel algal photobioreactor testbed will be used to further research dependencies for green algal growth. Two different environmental pressures were chosen to be maintained inside the photobioreactor. Specifically, 8.2 psia (56.5 kPa), which represents NASA's proposed exploration atmosphere, and 14.7 psia (101.3 kPa), which represents standard sea level pressure, were chosen. The algal solution will be taken from a lab culture in the log phase, diluted to a viable cell density of 10^6 cells/ml in Bolds Basic Medium (BBM) and inoculated into the photobioreactor. An air mixture will be created that consists of a partial pressure of 3.08 psia (21.2 kPa) oxygen, supplemented with a partial pressure of 0.15 psia (1.0 kPa) carbon dioxide. This correlates to an oxygen concentration of 21/38 % and a carbon dioxide concentration of 1/1.8 % at a total pressure of 14.7/8.2 psia (56.5/101.3 kPa). The balance will be filled with the inert gas nitrogen. The oxygen partial pressure is originating from the 21 % ambient oxygen concentration on Earth. The carbon dioxide partial pressure is derived from an optimum carbon dioxide concentration of 1 % at sea level pressure.

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The algal growth will be characterized by absorption measurements and cell counts on daily samples only. Algal metabolism will be continuously measured by carbon dioxide and oxygen concentration in the gas phase using a gas analyzer as well as dissolved oxygen concentration and pH value in the liquid phase using submergible probes. Temperature, light cycle, spectrum, intensity, and duration will be held constant at 30 °C, 12:12, cool white, 150 $\mu mol \cdot m^{-2} \cdot s^{-1}$, and 7 days respectively. The entire algal solution will be kept well mixed using sparging together with a magnetic stirrer. Experiments will be conducted in triplicates. The experiment will start with a 12-hour dark cycle to allow for adaptations of the algal cells. Additionally, metagenomics analysis will be conducted. Cell densities will be measured using a photometer for absorptivity measurements as well as a hemocytometer for more precise cell counts. Before sample taking, the culture will be well stirred so that a uniform distribution of algal cells in the culture can be assumed.

Table 4: Experiment variables for objective 3

Class	**Variable**
Independent Variable	total pressure
Dependent Variable	**continuous:** carbon dioxide concentration, oxygen concentration, pH, dissolved oxygen **initial and final measurement:** cell density
Constant Variable	temperature, light cycle, spectrum, carbon dioxide partial pressure, oxygen partial pressure, nutrient availability, duration
Confounding Variable	light intensity

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3.1.4 Objective 4 – Effect of altered oxygen concentration on population growth and metabolism of *Chlorella vulgaris* under reduced total pressure

It has been shown that increased oxygen concentration has a limiting influence on the algal growth under atmospheric pressures. The novel algal photobioreactor testbed will be used to research, if this observation is also true under a reduced pressure of 8.2 psia. The algal solution will be taken from a lab culture in the log phase, diluted to a viable cell density of 10^6 cells/ml in BBM and inoculated in the photobioreactor. The photobioreactor will be fed with a 1.8 % carbon dioxide concentration at 8.2 psia (56.5 kPa). The oxygen partial pressure will be held constant in two different cases. The first at 3.08 and the second at 2.79 psia. The remainder of the inlet air will be inert nitrogen. The oxygen partial pressure of 3.08 psia together represents Earth current condition. A partial pressure of 2.79 psia together with the absolute pressure of 8.2 psia (56.5 kPa) (resulting in an oxygen concentration of 34 %) represents the planned exploration atmosphere.

The algal growth will be characterized by absorption measurements and cell counts on daily samples only. Algal metabolism will be continuously measured by carbon dioxide and oxygen concentration in the gas phase using a gas analyzer, as well as dissolved oxygen concentration and pH value in the liquid phase using submergible probes. Temperature, light cycle, spectrum, intensity, and duration will be held constant at 30 °C, 12:12, cool white, 150 $\mu mol \cdot m^{-2} \cdot s^{-1}$, and 7 days respectively. The entire algal solution will be kept well mixed using sparging together with a magnetic stirrer. Experiments will be conducted in triplicates. The experiment

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will start with a 12-hour dark cycle to allow for adaptations of the algal cells. Additionally, metagenomics analysis will be conducted. Cell densities will be measured using photometer for absorptivity measurements as well as a hemocytometer for more precise cell counts. Before sample taking, the culture will be well stirred so that a uniform distribution of algal cells in the culture can be assumed.

Table 5: Experiment variables for objective 4

Class	Variable
Independent Variable	Oxygen concentration
Dependent Variable	**continuous:** carbon dioxide concentration, oxygen concentration, pH, dissolved oxygen **initial and final measurement:** cell density
Constant Variable	temperature, light cycle, spectrum, carbon dioxide partial pressure, total pressure, nutrient availability, duration
Confounding Variable	light intensity

3.1.5 Objective 5 – Effects of chemical contaminants on the health of algal cultures

Despite biological contamination that is expected to occur in the algal culture used in objectives 3 and 4, chemical contamination is the other relevant thread to the survival of algal cultures. Relevant spacecraft airborne contaminants are identified and their expected levels established from literature. From the stock culture in the exponential growth phase, samples are taken that are exposed to the contaminants

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in the specified levels and incubated under laboratory conditions and continuous illumination. Constant temperature, mixing, light intensity, spectrum, and atmosphere are ensured between the samples. Macroscopic observations and microscopic observations are performed to assess the health of the culture at the end of the experiment. Cell viability is assessed by a vital stain.

3.1.6 Objective 6 – First-order feasibility assessment of using an algal photobioreactor for spacecraft life support

An algal photobioreactor for biological life support will only be incorporated into a spacecraft ECLSS when its properties are shown to be superior compared to state-of-the-art systems. Those properties generally include mass, volume, power, cost, reliability, resupplies, and maintenance intervals, with robustness becoming more important as missions are conducted farther from Earth.

In this chapter, a first-order feasibility study to size an algal photobioreactor for a single crewmember in terms of air revitalization only is conducted. Based on the maximum experimentally reported oxygen evolution and carbon dioxide absorption values for algae, a minimum cell culture volume is established. Driven by the biological needs of this high-efficiency and high-density algal culture, critical photobioreactor design requirements for lighting, gas transfer, urine growth media, as well as continuous harvesting are established. Special consideration is given to constraints derived from a spacecraft implementation such as gravity, pre-existing mass streams, as well as resource efficiency. Based on these assumptions, a

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conceptual implementation of a peak performance, minimal size algal photobioreactor is presented.

The necessary mass, power, volume, and consumables required for such a bioregenerative system are calculated based on International Space Station (ISS) environmental conditions and from commercially available products such as light-emitting diodes (LED) and gas transfer membranes. Even though the envisioned photobioreactor in this analysis is sized for air revitalization purposes only, the impact of this system on food supplementation and water recycling is also estimated. Finally, the design concept is compared to current state-of-the-art air revitalization and urine processing technologies and values obtained in objectives 3 and 4. The feasibility to implement a sustainable photobioreactor into a spacecraft based on those functions alone is discussed. Potential mass savings are outlined, while implementation challenges and research gaps are identified.

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Table 6: Summary of research objectives

Obj.	Description	Chapter
1.*	Assess the influence of microgravity on algal growth based on past flight-experiments	4
2.	Develop a novel test bed for flow-through measurements of algal metabolism under altered pressure for bioregenerative life support applications	5
3.	Characterize the effect of varying total pressure on population growth and metabolism of *Chlorella vulgaris* at constant oxygen and carbon dioxide partial pressures	6
4.	Characterize the effect of altered oxygen concentration on population growth and metabolism of *Chlorella vulgaris* under reduced total pressure	-
5.*	Characterize the effect of chemical contaminants on the health of algal cultures	7
6.	Perform a first-order feasibility assessment of using an algal photobioreactor for spacecraft life support	8

* Objectives 2, 3, 4, and 6 are unchanged from the comprehensive examination. Objectives 1 and 5 were added in the course of the dissertation.

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4 Algae in microgravity

4.1.1 Introduction

Since the beginning of spaceflight, algae have been intensively studied for this purpose, even before the first human flew in space (Averner et al., 1984). Numerous factors can influence the growth of algae in a spacecraft cabin environment, with radiation and microgravity being the most unique to spaceflight (Tobias Niederwieser et al., 2018a). Outside of the cabin habitat, temperature extremes as well as the exposure to space vacuum must also be taken into account (Thirsk et al., 2009). In order to design an ECLSS utilizing biological systems, it is important to understand the response and adaptation of algae to these effects. As microgravity and the specific radiation environment cannot be fully replicated on Earth, it is especially important to conduct spaceflight experiments addressing those two parameters.

Previous reviews found in the literature either do not focus specifically on algae, cover a limited time span, or are specific to certain countries and their space programs (Alexandrov, 2016; Ang, 2004; Dickson, 1991; Harvey and Zakutnyaya, 2011; Kordyum, 1997). Therefore, to thoroughly document the quantity and quality of results already obtained, it is advantageous to list and analyze the prior spaceflight data comprehensively.

4.1.2 Material and methods

The data presented in this review article were mainly obtained through published literature. Flight details were extracted from the journal article or

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technical report on the flown experiment, where provided. Investigations that did not publish, or where a cited publication could not be found, are included using secondary information from flight manifests, earlier review papers, news articles, or books. All orbital flights to date that reported carrying some strain of algae, including green algae, euglenophytes and/or cyanobacteria, are listed in chronological order in Table 7 comprehensively.

An attempt was made to identify all experiments flown in space since the first known flight in 1960. Due to the wide variation in time, language, and organizations, data acquisition was challenging. Even though this work was done as thoroughly as possible, it cannot be guaranteed that the information presented here is comprehensive.

4.1.3 Results

The earliest verifiable record for algae flown in orbit was in 1960, together with a variety of other biological organisms, on board the Soviet Korabl-Sputnik 2 spacecraft – the 5th satellite ever launched into space. For this first mission, the algae cultures were grown in two groups for a total duration of 25 hours: the first on agar in darkness and the second in liquid media under periodic illumination. Even though a higher number of dead cells, as well as a decreased photosynthetic rate compared to ground controls was seen in post-flight analysis, it was concluded that algae can perform the main physiological processes of photosynthesis, growth, development, and reproduction in space (Semenenko and Vladimirova, 1961).

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Almost at the same time in the United States, a variety of studies with *Chlorella* cells were performed on the Discoverer spacecraft series for up to three days. These experiments launched photosynthetically inactive cells in darkness and performed microscopic and growth dynamic analyses during post-flight cultivation. Due to temperature variations, both in ground and flight cultures, it was difficult to draw any firm conclusions, but it was confirmed that algae were capable of surviving exposure to spaceflight conditions (Phillips, 1962; Ward and Phillips, 1968).

Encouraged by the initial success, Russian scientists launched a variety of *Chlorella* strains in the early 1960s on board the manned Vostok 5 and 6 missions, as well as the unmanned Cosmos 109 and 110 spacecraft. The Cosmos experiments employed photosynthetic inactive cells of up to five different *Chlorella* strains on agar, whereas the methods used in the Vostok experiments were not described in sufficient detail. All four flights conducted post-flight analysis using macro- and microcolony methods and reported no differences in survival or mutation frequency between flight and controls (Antipov et al., 1969; Shevchenko et al., 1967; Sisakyan et al., 1965). One notable experiment among those is the one performed on Cosmos 110. As all previous experiments had been relatively short (less than five days) and the effects of space environment are thought to be increased with longer mission durations, an experiment was flown on board the Cosmos 110 satellite for 22 days. This was almost three times longer than any prior experiment. Post-flight culturing showed a trend in delayed growth and reduced survival of the flight cultures but were

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statistically significant only for one strain of *Chlorella* (LARG-3) (Antipov et al., 1969).

The first experiment employing in-flight analysis on an active photosynthetic algal culture was planned by Ward et al. (1970) using *Chlorella sorokiniana* in liquid medium for 30 days. Unfortunately, the reactor developed a leak and exposed the algae to vacuum, which prevented any data collection (Ward et al., 1970).

Different from the previously described experiments conducted in Low Earth Orbit (LEO), the Zond missions flew on a trajectory around the Moon. This had a unique influence on the environment that the algae were exposed to, since it went outside the radiation-protecting Van Allen belts. The Zond missions around the Moon were hence exposed to both deep space radiation as well as to the radiation trapped in the Van Allen belts during the transit, making it difficult to distinguish between radiation and microgravity as the cause for potentially altered behavior. On the Zond 5, 6, 7, and 8 missions, *Chlorella vulgaris* cells were flown on agar in darkness for six or seven days. In post-flight culturing, no statistically significant differences were seen between flight and ground cultures using macro- and microcolony methods. Trends in survival and mutability were contradictory between the different experiments, which could have been due to unstable temperatures during the transport of the samples to the launch site. A very similar experimental setup and approach was consequently used by the same research group on board Cosmos 368, Soyuz 5, and Salyut 1 for a duration of up to 72 days with similar inconclusive results

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and failures (Anikeeva and Vaulina, 1971; Galkina and Aleksandrova, 1971; Galkina and Meleshko, 1975; Vaulina, E.N. and Moskvitin, 1975; Vaulina et al., 1971).

In 1970, Soyuz 9 carried the first successful attempt to grow a photosynthetically active culture in space for different times of 1, 6 and 14 days. Living samples were investigated back in the laboratory and analyzed by post-flight culturing for four days. It was reported that the duration of exposure to microgravity did not influence the cells sensitivity to flight factors, as no morphological or structural changes were seen in microscopic examinations. Post-flight cultivation dynamics followed the same patterns between the different flight groups as well as with the ground controls. The only difference observed using the microcolony methods was that productivity and sporulation were slightly decreased in the flight experiments, attributable to the increased death of cells exposed to the space environment (Moskvitin and Vaulina, 1975).

A total of five different algal experiments were conducted on the Salyut 6 space station, including different strains of *Chlorella* and *Scenedesmus obliquus*. The experiments were carried out both in liquid and on agar for durations between 4 and 18 days. All experiments employed post-flight analysis on the living algal cells and reported no changes between flight cultures and ground controls in microscopy, microcolony methods, and electron microscopy (Kordyum et al., 1979, 1980; NASA, 1988; Setlik et al., 1978).

Algal experiments were also conducted on board the Space Shuttle from the very beginning of the program. Axenic *Chlorella vulgaris* cultures were grown and fixed

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in-flight on STS-4 and an algae-kefir ecosystem with in-flight monitoring was flown on STS-51-G, but no resulting publications from either experiment were found in our literature search (Halstead and Dufour, 1986). In 1985, on board the German Spacelab mission (D-1), an experiment was carried out looking at the circadian rhythm of *Chlamydomonas reinhardtii*. This experiment was unique, as it successfully employed an in-flight measurement during active growth of the algae. Besides proving that the algae expressed a 24-hour period that was not significantly different from the ground controls during the 6.5 days of the experiment, it was also noted that the absorption amplitude of the space culture was larger. The amplitudes were in fact so large that they exceeded the range of the data acquisition system and therefore could not be recorded. It was hypothesized that the microgravity environment allowed the cells to maintain a more uniform suspension due to the lack of sedimentation. This potentially caused a more even illumination that distributed the light energy better to each cell. Additionally, some of the spaceflight cultures showed an increase in cell numbers, and higher survival rate than the control cultures on the ground (Mergenhagen and Mergenhagen, 1987).

The longest experiment using photosynthetically active algal cells in a spaceflight experiment to date was performed for a duration of 12 months on board the space station Mir. *Chlorella vulgaris* was grown on agar under illumination and fixed post-flight for cytological analysis. Observations under the electron microscope suggest that patterns in subcellular structural re-organization occurred in flight. This included reduced starch content, enlarged lipid drops, and increased vacuolization,

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as well as an increase in the size of mitochondria and the loss of the amylogenic coat around the pyrenoid. These changes suggest use of starch, and development of lipid storage associated with cell stress. It was also concluded that there were more types of organelle ultrastructure rearrangements observed within the flight cells that had been grown on solid medium than in cells grown on liquid or semi-liquid medium. The design of the experiment was such, however, that observed changes may have been due to media type rather than effects of spaceflight (Sytnik et al., 1992).

During the Cosmos missions in the late 80s and early 90s several algal experiments were conducted. Some of them again had failures due to temperature extremes, but most notably, heterotrophic experiments employing algae together with bacteria and fish demonstrated that it was possible to employ a small-scale life support system for durations of up to 13 days. The fish not only survived but reproduced during the experiment. Post-flight analysis performed on the algae in this three-component aquatic system showed some changes in the cell organization under electron microscopy. Perhaps most notable, the number of cells infected with bacteria was higher in the space populations, representing up to 3 % of the cells analyzed. Other changes observed, specifically, a decrease in number and size of starch grains in chloroplast stroma, more evident vacuolization of 10-16 % of the cell volume, a decrease in the amount of reserve polysaccharides and a reduction of the starch amounts in plastids were similar to results in monocultures of *Chlorella* in space (Connolly et al., 1994; Popova and Sytnik, 1996; Popova et al., 1989; Sychev et al., 1989).

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Chinese researchers started in the 90s to also perform experiments on algae in space. Their experiments attempted to actively culture algae in photobioreactors and perform in-flight measurements during the cultivation. Unfortunately, all experiments reported failures, either due to an incorrect light schedule, or spikes in the online optical density (OD) measurements which made analysis unreliable (G. . Wang et al., 2004; Wang et al., 2006; G. H. Wang et al., 2004).

Starting in 2005, Girardi et al. flew a variety of algal experiments involving *Chlamydomonas reinhardtii* as part of the Photo project (Bertalan et al., 2007; Giardi et al., 2013; Pezzotti and Giardi, 2011; Vukich et al., 2012). Most notably, in 2005 and 2007, a wild type and 4 different mutants of *Chlamydomonas reinhardtii* were flown on board the Russian Foton M2 and M3 missions. The mutants differed in their amino acid substitutions in the D1 protein, important for photosynthesis. Cultures were artificially illuminated and the photosynthetic performance was recorded in-flight using fluorescence measurements. While the wildtype together with two mutants showed reduced photosynthetic activity, two mutants showed increased photosynthetic efficiencies in space, and were able to re-grow once back on earth. Modern techniques including RNA and protein extraction, oxygen evolution measurements, as well as excitation pressure have been measured on the post-flight cultivation three days after landing. The authors confirmed the important role the D1 protein plays in stabilizing and enhancing Photosystem II function under extreme environments (Giardi et al., 2013).

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All the experiments described so far were performed within the pressurized spacecraft cabin, including artificial conditions imposed on pressure, temperature, and lighting. An experiment in 2008, however, stored several rock samples with microbial communities contained on them using the EXPOSE-E facility outside of the ISS for 18 months. Samples were kept in darkness but exposed to space vacuum and temperature extremes. Two green algae (*Chlorella* and *Rosenvingiella spp.*) as well as a cyanobacterium (*Gloeocapsa sp.*) from the microbial community were reported to have survived the space conditions (Cockell et al., 2011).

In 2011, German researchers flew an artificial ecosystem on board the Chinese Shenzhou-8 satellite consisting of *Euglena gracilis* (a Euglenophyte) and snails. The algae sufficiently produced oxygen and absorbed carbon dioxide to keep the snails alive during the 17-day mission. At this time, post-flight RNA analysis is still in progress for further results on the biological response (Preu and Braun, 2014).

On the final flight of the Space Shuttle Endeavour (STS-134) in 2011, *Chlamydomonas reinhardtii* cells were flown both inside the cabin as well as in a hermetically sealed container in the cargo bay. The latter exposed a selection of viable algal mutants in a pressurized compartment to the space radiation through a transparent cover. Post-flight analysis using PCR, oxygen evolution measurements, and fluorescence measurements revealed that the mutants displayed a higher photosynthetic performance and a faster rate of re-growing compared to the parental strain indicating a higher capacity of stress recovering (Vukich et al., 2012). In contrast, the internal experiment called “Night Vision” used immobilized eyespots

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and macular pigments extracted from *Chlamydomonas reinhardtii*, as a model organism for the human retina. Results obtained from the effect of cosmic radiation on the eyespots were thought to allow adapting future crew nutrition to prevent or mitigate damage to the human eye during spaceflight. Results from this experiment however have not been found in the published literature (Pezzotti and Giardi, 2011).

In 2016, another experiment stored various specimens of slightly desiccated algae on the outside of the ISS for 16 months. The specimens were from two strains of algae: the green alga CCCryo 101-99 of *Sphaerocystis sp.* and the cyanobacterium CCCryo 213-06 of *Nostoc sp.* This time, the samples were exposed not only to space vacuum and temperature extremes, but also to high levels of solar radiation. After their return from the ISS, all specimens except one developed into new populations during post-flight cultivation and the investigators are planning to examine the DNA for understanding the underlying characteristics (Baqué et al., 2017).

A variety of educational experiments performed by students have also been conducted using different strains of algae on board the ISS in 2016 and 2017, but no results have been found in the published literature (NASA, 2017a, 2017b, 2017c, 2017d).

A German experiment that is currently planned to launch in 2018 will employ a photobioreactor intended to demonstrate the life support functionality of absorbing carbon dioxide and evolving oxygen on board the ISS. *Chlorella vulgaris* will be fed with a carbon dioxide stream from the newly deployed Advanced Closed Loop System and monitored continuously in-flight (Keppler et al., 2017).

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4.1.4 Discussion

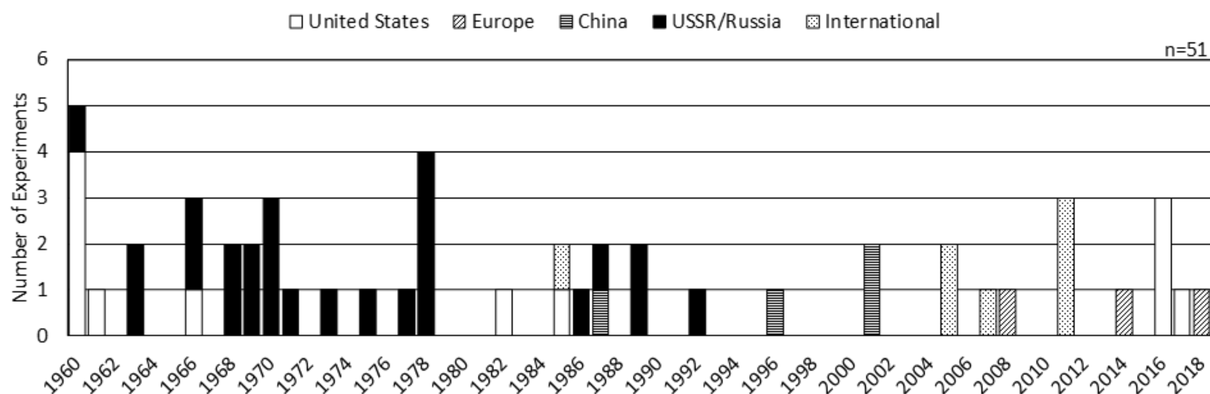

Figure 10: Timeline showing number of algal experiments flown in space

In this review, a total of 51 algal experiments flown in space have been identified (Figure 10) starting from the early days of space flight in 1960 through planned experiments in 2018. From this literature, it can be concluded that algae have been widely used as model research organisms flown in space.

As can be seen in Figure 11, species that have been investigated are mainly from the genus *Chlorella* (*Chlorella vulgaris*, *Chlorella pyrenoidosa*, *Chlorella sorokiniana*, *Chlorella ellipsoidea*), but other green algae species such as *Chlamydomonas reinhardtii*, *Scenedesmus obliquus*, *Rosenvingiella sp.*, and *Sphaerocystis sphaerocystiformis* have also been flown. Of the cyanobacteria, *Nostoc sphaeroides, Gloeocapsa sp.* and *Anabaena siamensis* have been employed in spaceflight experiments. Additionally, *Euglena gracilis,* a euglenophyte, was flown in one instance. Algae in both photosynthetically-active and inactive states have been shown to survive exposure to a variety of spaceflight cabin conditions including radiation and weightlessness. Most importantly, it has been demonstrated that even direct exposure to the vacuum environment of space can be withstood by inactive

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algae, which is important for stored inoculation cultures during emergencies such as a depressurization or during uncrewed time periods of a mission. This ability can greatly enhance the reliability of bioregenerative life support systems in general.

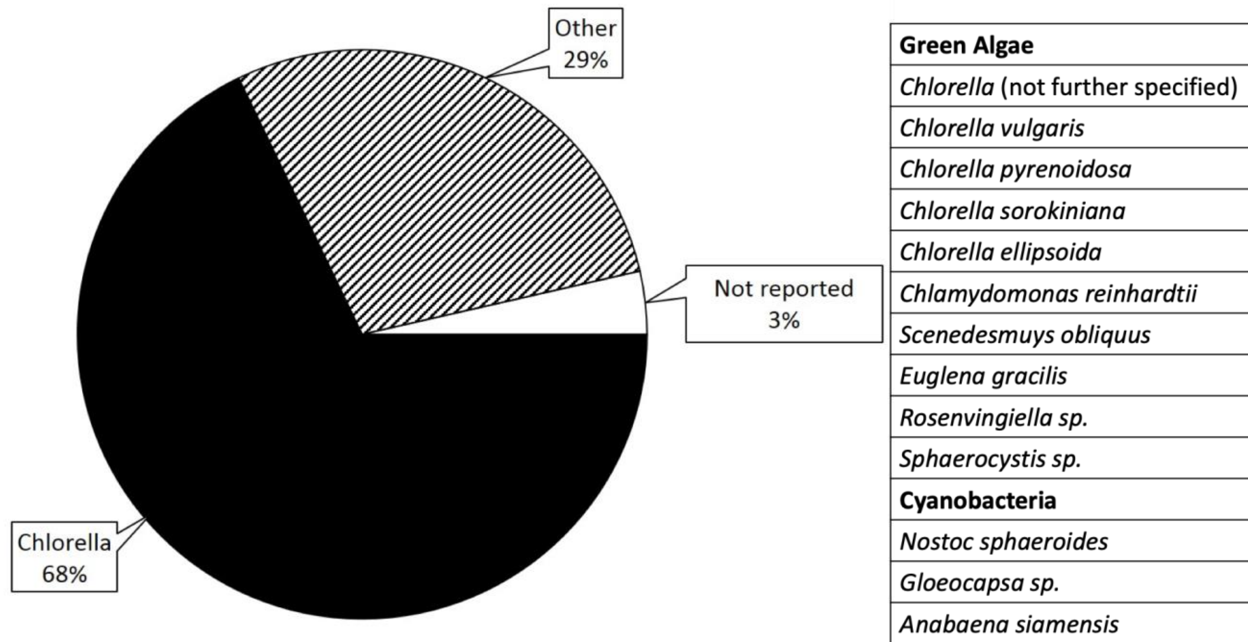

Figure 11: Algal species flown in space

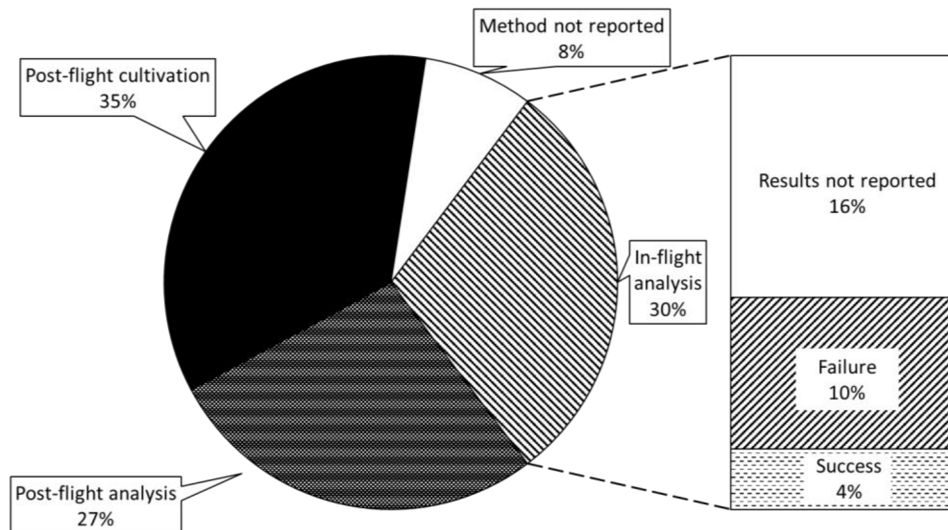

Figure 12: Analytical methods used in flight experiments

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Even from this relatively large number of algal spaceflight experiments conducted thus far, data are still quite limited. For example, as indicated in Figure 12, no experiments to date have successfully reported in-flight growth rate of algae (as opposed to pre- and post-flight cell counts), as they have either had a technical failure, used experimental designs that precluded attributing differences to space conditions, or the resulting data could not be found in the published literature. Only two experiments have successfully collected in-flight measurements. Both of those were collected using the same instrument (Photo II), targeting photosynthetic efficiency using fluorescence (Pezzotti and Giardi, 2011). Differences in photosynthetic efficiencies have been found between different mutants. Most other experiments have fixed their cultures post-flight (at the earliest several hours after landing) or have conducted their analysis on viable post-flight cultures. This approach, however, allows the algae to readapt to 1g from the intended growth conditions under microgravity, thus confounding isolation of gravity as the independent variable. That the results obtained from post-flight analysis should be reviewed with great care can be emphasized by two observations: First, the reported outcomes are often contradictory and have almost never shown statistically-significant differences. This could be due to readaptation of the algal cells to Earth gravity before analysis as noted above, and therefore present a synchronization issue with the 1g ground controls. Second, the experiment by Mergenhagen and Mergenhagen (1987) described above using in-flight data analysis showed noticeable differences in OD during microgravity, hinting at altered behavior of algal cells in

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space, but not definitively concluding this as such (Mergenhagen and Mergenhagen, 1987). Results with such obvious differences between flight and ground groups, however, have not been recorded in any of the other experiments.

The simplicity of the early experiments, especially regarding the frequent lack of thermal control either during the flight or during ground handling, exposed the algae to a wide range of temperatures outside their optimum growth conditions that may have affected the outcomes. Since it is known that temperature affects the photosynthetic activity of algae (Cassidy, 2011; Chinnasamy et al., 2009; Converti et al., 2009; Dauta et al., 1990), the results from those experiments often allow no more conclusions to be drawn than the fact that algae can survive the space environment in general. This is also true for making relative comparisons between flight and ground control as they were often exposed to numerous different parameters.

One issue that should be noted is that the cells flown in orbit are exposed to both microgravity and increased radiation. Most of the early flight experiments described here were conducted in LEO, therefore within the Van Allen belts, of relatively short duration, and inside the shielding of a spacecraft, so radiation effects are likely to be minimal and reduced gravity is dominant. However, on experiments conducted during the long-duration missions or the flyby missions around the Moon, the effect of radiation relative to the effect of microgravity on the experiment is not clear.

4.1.5 Conclusions

So far, it has been shown that the basic biological processes associated with algal growth, photosynthesis, and respiration can function in space for durations of up to

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12 months. However, dynamic culture behavior in the spacecraft environment needs further assessment and quantification and, in particular, the effects of radiation have not yet been sufficiently examined. Modern analytical tools such as RNA sequencing together with in-flight fixation of samples should help to better characterize the overall biological response of the cells. Results from these prior and future experiments will be helpful towards designing a BLSS and selecting the most suitable algal strains for long-term human spaceflight missions. Researching algal adaptations to the space environment can potentially even be used towards developing new genetically-modified algal species that have specific characteristics desirable for terrestrial applications as well as for space. It can be concluded that despite the large number of algal experiments conducted in space to date, our understanding of the effects of microgravity and radiation on algae remains limited.

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Table 7: Algal experiments flown in space

Year	Author	Spacecraft	Algal strain	State	Time	Analysis	Failures	Result
1960	Semenenko and Vladimirova (1961) (Semenenko and Vladimirova, 1961)	Korabl-Sputnik 2	*Chlorella pyrenoidosa* H.Chick	axenic, liquid (agar) medium, periodic illumination (darkness)	1 d	post-flight		No significant lethal or irreversible changes could be observed in the main physiological processes such as photosynthesis, growth, development or reproduction.
1960	Phillips (1962) (Phillips, 1962)	Discoverer 17	*Chlorella ellipsoidea* SAM 127	axenic, modified liquid Kratz medium, darkness	2 d	post-flight cultivation	no growth of ground controls	Photosynthetic organisms are capable of living and retaining viability in actual space environments.
1960	Ward and Phillips (1968) (Ward and Phillips, 1968)	Discoverer 17	*Chlorella pyrenoidosa* SAM 127	axenic, liquid medium, darkness	2 d	post-flight cultivation	no flight temperature recording (21±5 °C) ground control temperatures >50 °C	No adverse effects on viability, growth rate, or morphology were observed during post-flight comparison with controls.
1960	Ward and Phillips (1968) (Ward and Phillips, 1968)	Discoverer 18	*Chlorella pyrenoidosa* SAM 127	axenic, liquid medium, darkness	3 d	post-flight cultivation	no flight temperature recording (21±5 °C)	No adverse effects on viability, growth rate, or morphology were observed during post-flight comparison with controls.
1960	Ward and Phillips (1968) (Ward and Phillips, 1968)	Discoverer 29	*Chlorella pyrenoidosa* SAM 127	axenic, liquid medium, darkness	2 d	post-flight cultivation	no flight temperature recording (21±5 °C) damage obtained during heat sealing	No adverse effects on viability, growth rate, or morphology were observed during post-flight comparison with controls.

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Year	Author	Spacecraft	Algal strain	State	Time	Analysis	Failures	Result
1961	Ward and Phillips (1968) (Ward and Phillips, 1968)	Discoverer 30	*Chlorella pyrenoidosa* SAM 127	axenic, liquid medium, darkness	2 d	post-flight cultivation	no flight temperature recording (21±5 °C)	No adverse effects on viability, growth rate, or morphology were observed during post-flight comparison with controls.
1963	Sisakyan et al. (1965) (Sisakyan et al., 1965)	Vostok 5	*Chlorella* (several strains)	NR	5 d	post-flight	NR	No appreciable influence of flight conditions on survival and frequency of mutations of the bulk of strains.
1963	Sisakyan et al. (1965) (Sisakyan et al., 1965)	Vostok 6	*Chlorella* (several strains)	NR	3 d	post-flight	NR	No appreciable influence of flight conditions on survival and frequency of mutations of the bulk of strains.
1966	Shevchenko et al. (1967) (Shevchenko et al., 1967)	Cosmos 109	*Chlorella* (LARG-1, LARG-3, LARG-5)	axenic, agarized Tamiha medium, darkness	8 d	post-flight cultivation	NR	No significant differences in the occurrence frequency of visible mutations in reaction cell cultures between test and control material.
1966	Antipov et al. (1969) (Antipov et al., 1969)	Cosmos 110	*Chlorella* (LARG-1, LARG-3, LARG-5, U158, U-125)	axenic, agarized Tamiha medium, darkness	22 d	post-flight cultivation	NR	No significant differences in the occurrence frequency of visible mutations in reaction cell cultures between test and control material.
1966	Ward et al. (1970) (Ward et al., 1970)	OV1-4	*Chlorella sorokiniana*	axenic, liquid Knop's medium, 12:12 illuminated	30 d	in-flight	leak in gas chamber and loss of pressure	No data due to failure.
1968	Vaulina et al. (1971) (Vaulina et al., 1971)	Zond 5	*Chlorella vulgaris* Bejer (LARG-1, LARG-3)	axenic, agarized Tamiha medium, darkness	6 d	post-flight cultivation	several temperature drops (even below freezing) during transportation to launch site	Considerable decrease of survival and increase in the frequency of mutation in flight. Marked depression of cellular development.

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Year	Author	Spacecraft	Algal strain	State	Time	Analysis	Failures	Result
1968	Vaulina et al. (1971) (Vaulina et al., 1971)	Zond 6	*Chlorella vulgaris* Bejer (LARG-1)	axenic, agarized Tamiha medium, darkness	6 d	post-flight cultivation	several temperature drops (even below freezing) during transportation to launch site	Marked depression of cellular development.
1969	Anikeeva and Vaulina (1971) (Anikeeva and Vaulina, 1971)	Soyuz 5	*Chlorella vulgaris* Bejer (LARG-1)	axenic, agarized Tamiha medium, darkness	3 d	post-flight cultivation	several temperature drops (even below freezing) during transportation to launch site	All of the impairments were caused not by flight factors, but by conditions accompanying the experiment.
1969	Vaulina et al. (1971) (Vaulina et al., 1971)	Zond 7	*Chlorella vulgaris* Bejer (LARG-1)	axenic, agarized Tamiha medium, darkness	6 d	post-flight cultivation	several temperature drops (even below freezing) during transportation to launch site	Tendency toward increase of survival and a decrease of mutability in the culture. Marked depression of cellular development.
1970	Moskvitin and Vaulina (1975) (Moskvitin and Vaulina, 1975)	Soyuz 9	*Chlorella vulgaris* strain 60	axenic, agarized nutrient medium, constant illumination	1, 6, 14 d	post-flight cultivation	NR	There is an insignificant change in sensitivity of the cells to the effect of flight factors depending on the duration of their exposure in an active state.
1970	Galkina and Aleksandrova (1971) (Galkina and Aleksandrova, 1971)	Cosmos 368	*Chlorella pyrenoidosa Sp.*	axenic, agarized Tamiya medium, darkness	6 d	post-flight cultivation	brief rise in temperature to 35 °C	No significant changes in cell morphology and photosynthetic activity.
1970	Vaulina and Moskvitin (1975) (Vaulina, E.N. and Moskvitin, 1975)	Zond 8	*Chlorella vulgaris* Bejer (LARG-1)	axenic, agarized Tamiha medium, darkness	7 d	post-flight cultivation	NR	Flight conditions had a negative effect on the viability and mutability of *Chlorella vulgaris* cells but were statistically unreliable.

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Year	Author	Spacecraft	Algal strain	State	Time	Analysis	Failures	Result
1971	Galkina and Meleshko (1975) (Galkina and Meleshko, 1975)	Salyut 1	*Chlorella vulgaris* Bejer (LARG-1)	axenic, agarized Tamiha medium, darkness	72 d	post-flight cultivation	NR	No influence in the basic physiological parameters (rate of growth and reproduction, cell size and population structure, chlorophyll content, cell viability).
1973	NR	Soyuz 13	*Chlorella*	NR	NR	NR	NR	NR
1975	NR	Salyut 4	*Chlorella*	NR	NR	NR	NR	NR
1977	Sychev and Galkina (1986) (NASA, 1988)	Salyut 6	*Chlorella*	axenic, liquid medium, illuminated	4-18 d	post-flight cultivation	NR	Time to generate individuals, number of autospores forming, time ratios of developmental phase of individuals were normal.
1978	Kordyum et al. (1980) (Kordyum et al., 1980)	Salyut 6 (Soyuz 27)	*Chlorella pyrenoidosa* (LARG-1)	axenic, semiliquid mineral/glucose medium, darkness	5 d	post-flight	NR	The ultrastructural cellular organization of the flight and control cultures were similar. This testifies to normal cell function.
1978	Kordyum et al. (1979) (Kordyum et al., 1979)	Salyut 6 (Soyuz 27)	*Chlorella vulgaris* (LARG-1)	axenic, semiliquid mineral/glucose medium, darkness	5 d	post-flight	NR	The ultrastructural cell organization does not reveal essential reconstruction.
1978	Setlik et al. (1978) (Setlik et al., 1978)	Salyut 6 (Soyuz 28)	*Chlorella vulgaris*	heterotrophic growth, mineral medium, darkness	8 d	post-flight	NR	No change in growth rate or population characteristics.
1978	Setlik et al. (1978) (Setlik et al., 1978)	Salyut 6 (Soyuz 28)	3 *Chlorella* strains and *Scenedesmus obliquus*	axenic, mineral medium, darkness	8 d	post-flight	NR	No change in growth rate or population characteristics.
1982	NR	STS-4	*Chlorella vulgaris*	NR	NR	in-flight	NR	NR
1985	NR	STS-51-G	*Chlorella*	algae-kefir	5 d	in-flight fixation	NR	NR

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Year	Author	Spacecraft	Algal strain	State	Time	Analysis	Failures	Result
1985	Mergenhagen and Mergenhagen (1987) (Mergenhagen and Mergenhagen, 1987)	STS-61-A (D-1)	*Chlamydomonas reinhardtii*	axenic, liquid medium, darkness	6 d	in-flight	amplitudes outside of sensor measurement range	Survival rate was higher in space than on the ground.
1986	Sytnik et al. (1992) (Sytnik et al., 1992)	Mir	*Chlorella vulgaris* (LARG-1)	axenic, solid agarized medium, illuminated	5, 30 d, 12 m	post-flight fixation	NR	Comparative cytological analysis has revealed general regularities of rearrangements of the submicroscopic organization.
1987	Zheng-Chang (1988) (Zhen-Chang, 1988)	FSW 1-1	NR	NR	5 d	NR	NR	NR
1987	Sychev et al. (1989) (Sychev et al., 1989)	Bion-8 (Cosmos 1887)	*Chlorella vulgaris* Beijer	algae-bacteria-fish	13 d	NR	NR	NR
1989	Popova et al. (1989) (Popova et al., 1989)	Bion-9 (Cosmos 2044)	*Chlorella vulgaris* (LARG-1)	algae-microorganism-fish, liquid, illuminated	13 d	post-flight fixation	NR	An increase in the number of *Chlorella* cells infected by bacteria was shown in the experimental variant. No considerable differences were established in the growth characteristics of the experimental and control populations. A comparative cytological analysis revealed general regularities of organelles in *Chlorella* cells cultivated under space flight condition in the uni- and multicomponent systems.
1989	Connolly et al. (1994) (Connolly et al., 1994)	Bion-9 (Cosmos 2044)	*Chlamydomonas reinhardtii*	axenic, solid agarized medium, illuminated	14 d	post-flight fixation	NR	Some changes in distribution of the main cell organelles.

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Year	Author	Spacecraft	Algal strain	State	Time	Analysis	Failures	Result
1992	Popova and Sytnik (1996) (Popova and Sytnik, 1996)	Bion-10 (Cosmos 2229)	*Chlorella vulgaris* Bejer (LARG-1)	axenic, solid agarized medium, darkness.	12 d	post-flight fixation	temperature rise to 30 °C before landing	An increase of mitochondria and their cristae size, as well as an increase of the total volume of mitochondrion per cell were established. The decrease of the starch amount in the plastid stroma and the electron density of the latter was also observed.
1996	Wang et al. (2006) (Wang et al., 2006)	Chinese retrievable satellite	*Anabaena siamensis* FACHB 799	axenic, liquid BG-11 medium, 12:12 illuminated	15 d	in-flight	light schedule wrong in spaceflight	No data due to failure.
2001	Wang et al. (2004) (G. . Wang et al., 2004)	Shenzhou-2	*Nostoc sphaeroides* Kütz	axenic, liquid basal medium, 12:12 illuminated	7 d	in-flight	experiment errors, spikes in microgravity	No data due to failure.
2001	Wang et al. (2004) (G. H. Wang et al., 2004)	Shenzhou-2	*Chlorella pyrenoidosa* FACHB 415	algae-snail, liquid medium, 12:12 illuminated	7 d	in-flight	spikes in microgravity OD measurements	No data due to failure.
2005	Giardi et al. (2013) (Giardi et al., 2013)	Foton-M2	*Chlamydomonas reinhardtii*	axenic, solid TAP agar medium, 7:17 illuminated	16 days	in-flight post-flight cultivation	NR	Due to ionizing radiation, some of the mutants showed an increased oxygen evolution capacity compared to the reference strain.
2005	Bertalan et al. (2007) (Bertalan et al., 2007)	Foton-M2	*Chlamydomonas reinhardtii*	axenic, solid TAP agar medium, ambient lighting	16 days	post-flight	NR	Stimulation of the oxygen evolution activity as well as increased cell size and extended stage of active culture growth in space
2007	Pezzotti et al. (2011) (Pezzotti and Giardi, 2011)	Foton-M3	*Chlamydomonas reinhardtii*	axenic, solid TAP agar medium, 7:17 illuminated	12 days	in-flight post-flight cultivation	NR	Due to ionizing radiation, some of the mutants showed an increased oxygen evolution capacity compared to the reference strain.
2008	Cockell et al. (2011) (Cockell et al., 2011)	ISS	*Chlorella, Rosenvingiella spp., Gloeocapsa sp.*	microbial community, ephilithic, darkness	18 m	post-flight	NR	Preserved cell morphology, while cells were bleached and carotenoids were destroyed.

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Year	Author	Spacecraft	Algal strain	State	Time	Analysis	Failures	Result
2011	Preu and Braun (2013) (Preu and Braun, 2014)	Shenzhou-8	*Euglena gracilis*	algae-snail, liquid medium, constant illumination	17 d	in-flight	NR	NR
2011	Vukich et al. (2012) (Vukich et al., 2012)	STS-134	*Chlamydomonas reinhardtii*	axenic, solid TAP agar medium, ambient lighting	16 d	post-flight cultivation	NR	Higher photosynthetic performance in space
2011	Pezzotti et al. (2011) (Pezzotti and Giardi, 2011)	STS-134	*Chlamydomonas reinhardtii*	axenic, solid TAP agar medium, 7:17 illuminated	16 d	post-flight	NR	NR
2014	Leya et al. (2017) (Baqué et al., 2017)	ISS	*Sphaerocystis sp.* CCCryo 101-99 and *Nostoc sp.* CCCryo 213-06	slightly desiccated at EXPOSE facility	16 m	post-flight cultivation	NR	After their return from the ISS almost all samples developed into new populations.
2016	NR	ISS	NR	axenic, semisolid agar medium, 11:13 illuminated*	30 d*	in-flight*	NR	NR
2016	NR	ISS	*Chlorella vulgaris**	axenic, liquid medium, illuminated*	NR	in-flight*	NR	NR
2016	NR	ISS	*Chlorella vulgaris**	axenic, agarized medium, 12:12 illuminated*	24 d*	in-flight*	NR	NR
2017	NR	ISS	*Chlorella vulgaris* and *Chlamydomonas reinhardtii**	NR	24 d*	in-flight*	NR	NR
2018*	Keppler et al. (2017) (Keppler et al., 2017)	ISS*	*Chlorella vulgaris**	modified diluted seawater nitrogen medium, constant illumination*	NR	in-flight*	NR	NR

** - proposed data taken from announcement; NR – not reported*

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4.2 Resultant publications and presentations

Journal Papers

1. **T. Niederwieser**, P. Kociolek, and D. Klaus, "A review of algal research in space," Acta Astronaut., 146, pp. 359–367, 2018.

Conference Papers (non-refereed)

1. **T. Niederwieser**, and D. Klaus, "Algal research in space," in Int. Astronaut. Congr. Adelaide, Australia, 2017 (IAC-17.A1.7.7x36885)

Presentations

1. 68th International Astronautical Congress (IAC), "Algal research in space" Adelaide, Australia, 2017.

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5 Development of an algal photobioreactor for research with altered atmospheric pressure and composition

In order to implement an algal photobioreactor into closed space habitats, further characterizations have to be conducted. This includes studying the effect of radiation, microgravity, contamination, and air composition on the algal growth and metabolism. As NASA has proposed an exploration atmosphere of 8.2 psia (56.5 kPa) and 34 % oxygen for future space habitats (Norcross et al., 2013), the effects of reduced pressure on algae is of particular interest (Wagner et al., 2016). A variety of algal photobioreactors have been developed and reviewed in the past (Borowitzka and Moheimani, 2013; Carvalho et al., 2006; Chaumont, 1993; Janssen et al., 2003; Lehr and Posten, 2009; Ugwu et al., 2008). Designs range from open raceway ponds, over flat-panel and tubular photobioreactors, to cylindrical bubble-column reactors. While each reactor type is advantageous for a specific application (for example mass production, research project, low cost, or low footprint) none of the reactors is suited to perform experiments under altered atmospheric pressure and composition. The ability to both increase and decrease pressure, as well as altering the gas composition while scientifically measuring algal metabolism and growth poses additional constraints on a photobioreactor design which are otherwise not needed. In this project, an algal photobioreactor is therefore developed that is capable of simulating the low pressure and high oxygen fraction environment of the proposed exploration atmosphere for studies on the algal performance in laboratory settings.

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5.1 Materials and methods

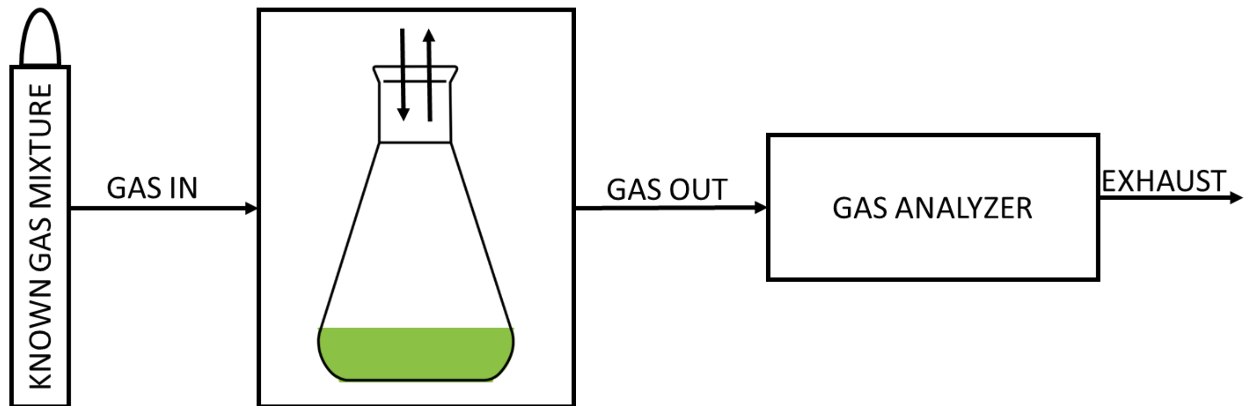

Figure 13: Flow-through experimental test setup schematic

Published algal studies found in the literature that were aimed at characterizing the effect of reduced total pressure were interested in the biomass production of algae (Orcutt et al., 1970; Qin et al., 2014a) and did not measure the oxygen evolution or carbon dioxide fixation rate. For life support applications, however, the algal metabolism is of interest, making the typical experimental setups insufficient. Therefore, the newly developed test setup follows a flow-through approach as depicted in Figure 13. A premixed gas mixture with known composition is fed into the variable-pressure photobioreactor that allows gas to exchange between the liquid and gaseous phase. The continuous gas supply also pushes the overflowing gas mixture out of the photobioreactor into the gas analyzer. Analysis of carbon dioxide and oxygen compositions at the gas outlet allows for performance calculations of the algae. This flow-through system has the advantage of exposing the algae to a steady state mixture from the inlet that does not get altered over time by the algal metabolism, as it is the case with hermetically sealed, batch-style photobioreactors. All metabolic activities can be measured at the outlet relative to the known input gas.

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5.1.1 Requirements

Table 8: Optimum growth conditions for Chlorella vulgaris

Variable	**Value**
Temperature	30 °C (Chinnasamy et al., 2009; Converti et al., 2009; Dauta et al., 1990)
Light spectrum	400 – 700 nm (Ravelonandro et al., 2008; Yeh et al., 2010)
Light intensity (within PAR range)	300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Bhola et al., 2011; Degen et al., 2001; Hassan et al., 2015; Huang et al., 2016)
Light cycle	12/12 (day/night) (Khoeyi et al., 2012)
Growth media type	Bolds Basic Medium (BBM) (Sharma et al., 2011)

To understand the influence of the gas composition on algae, the remaining environmental parameters have to be kept in known, and for their peak performance, controlled to their optimal ranges to not become a growth limiting factor. Environmental parameters affecting the growth of *Chlorella vulgaris* have been identified in previous studies (Alexandrov, 2016; Daliry et al., 2017; Rendon, 2014; Sharma et al., 2012). The variables with their selected correlating values are shown in Table 8. These values represent precise set points instead of minimum or maximum parameters. As shown in the example of light intensity, algae respond proportionally to low light levels during the light limited phase. At light levels

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ranging from 200-300 $\mu mol \cdot m^{-2} \cdot s^{-1}$, they are entering a plateau due to light saturation and are growth limited by other factors. Above 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$, they enter the light inhibited phase, where growth rate rapidly drops off. A similar behavior is also seen with temperature.

Table 9: Required gas input composition ranges

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

As the purpose of this experimental setup is to characterize the metabolism of algal cells in a photobioreactor under varying cabin atmosphere conditions, the testbed had to be capable of providing the input parameters in a wide range of gas compositions and pressures. Oxygen, carbon dioxide and nitrogen concentrations must be provided in ranges preferably between 0 to 100 % at standard sea level conditions. This equals a partial pressure range of 0 – 101.3 kPa for each gas, which is shown in Table 9. Specifically for oxygen, this requirement was limited to 21.3 kPa for flammability concerns. As a special requirement to incorporate NASA's proposed exploration atmosphere, the reactor was designed to be able to operate at total pressures between 56.5 and 101.3 kPa (8.2 and 14.7 psia).

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5.1.2 Design

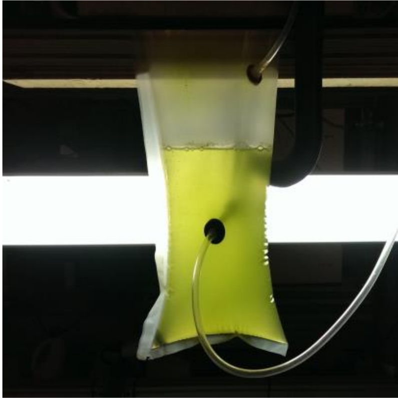
Figure 14: Gas-permeable bag photobioreactor

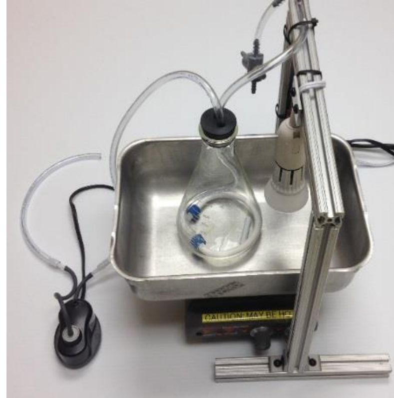
Figure 15: Erlenmeyer flask photobioreactor

Figure 16: Pressure tank photobioreactor

Several different designs were experimentally compared with each other. A gas-permeable bag approach (Figure 14) showed too large diffusion rates for both carbon dioxide and oxygen across the membrane to obtain useful data. Erlenmeyer flasks (Figure 15), which are commonly used to culture algae, do not provide pressure tight interfaces and showed unacceptably high leak rates. In a pressure tank housing (Figure 16), the reactor proved difficult to handle operationally, as the entire pressure tank had to be frequently opened to troubleshoot sensors, which required stopping the experiment. Additionally, condensation on the electronics within the pressure tank proved to be a challenge. Through the experience gained from these concepts, a benchtop photobioreactor was designed for the purpose of collecting metabolic data under altered pressures as described in the following paragraphs.

5.1.2.1 Mechanical design

To maintain comparability with previous studies (De Morais and Costa, 2007; Mata et al., 2010; Nauha and Alopaeus, 2013; Scarsella et al., 2010), a tubular photobioreactor design, as shown in Figure 17, was chosen. The central element of

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the design setup, as shown in Figure 18, is an acrylic reactor in a cylindrical shape which has an internal volume of 3.0 liters and is designed to withstand both positive and negative pressures of up to +18 kPa and -28 kPa. This is necessary to perform studies in the absolute pressure range of 56.5 kPa (exploration atmosphere) to 101.3 kPa (standard sea level), while accounting for an atmospheric pressure in Boulder of about 85 kPa due to elevation.

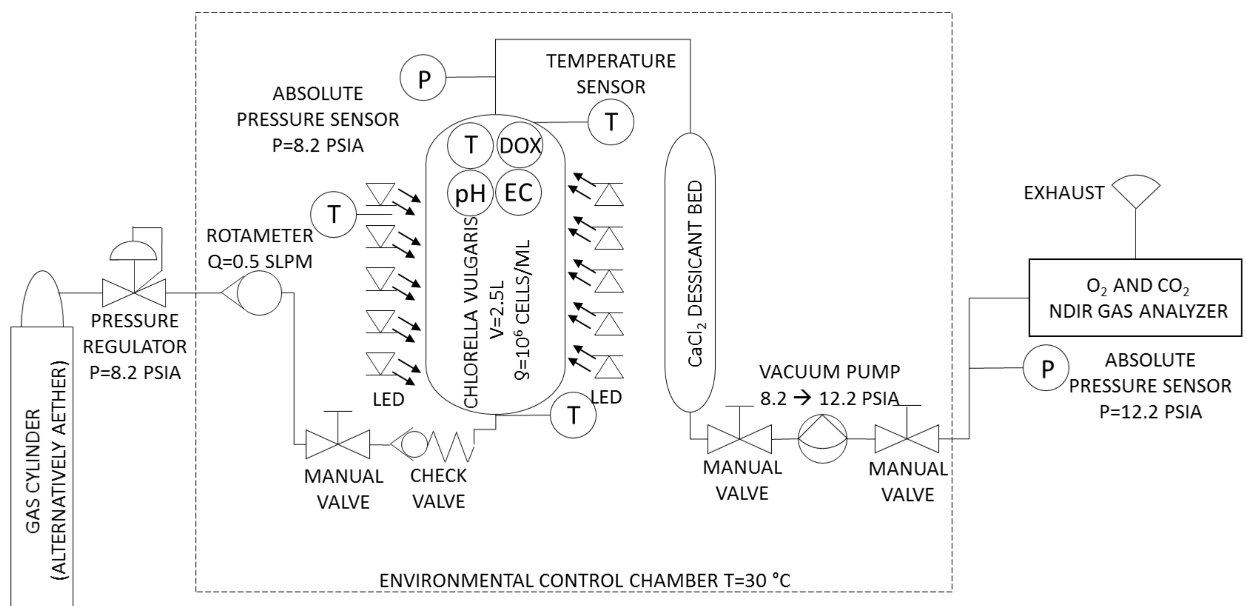

Figure 17: Piping and Instrumentation Diagram of test setup

The top and bottom of the cylinder are circular disks with integrated O-rings that are kept in place by two internal retaining rings. This allows for fast assembly and disassembly of the reactor to reach the internal surfaces for cleaning purposes. Circling around the photobioreactor, white light emitting diodes (LED) are arranged uniformly over the entire height and deliver a light intensity of 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ to the surface of the photobioreactor. White LEDs where used to replicate Earth lighting conditions for the crew. If power is a limiting factor, blue and red LEDs could be used

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instead to achieve slightly higher energy conversion rates. The components of the test setup between the rotameter and the vacuum pump are within an environmental chamber that controls the temperature to a constant 30 °C. This makes the experiment independent of any day/night cycles of the temperature within the lab. Additionally, the air stream within the environmental chamber cools the LED's and prevents a temperature change of the algal solution due to the light cycle. The bottom plate has an inlet in the center, where a constant air stream of 0.5 l/min is inserted.

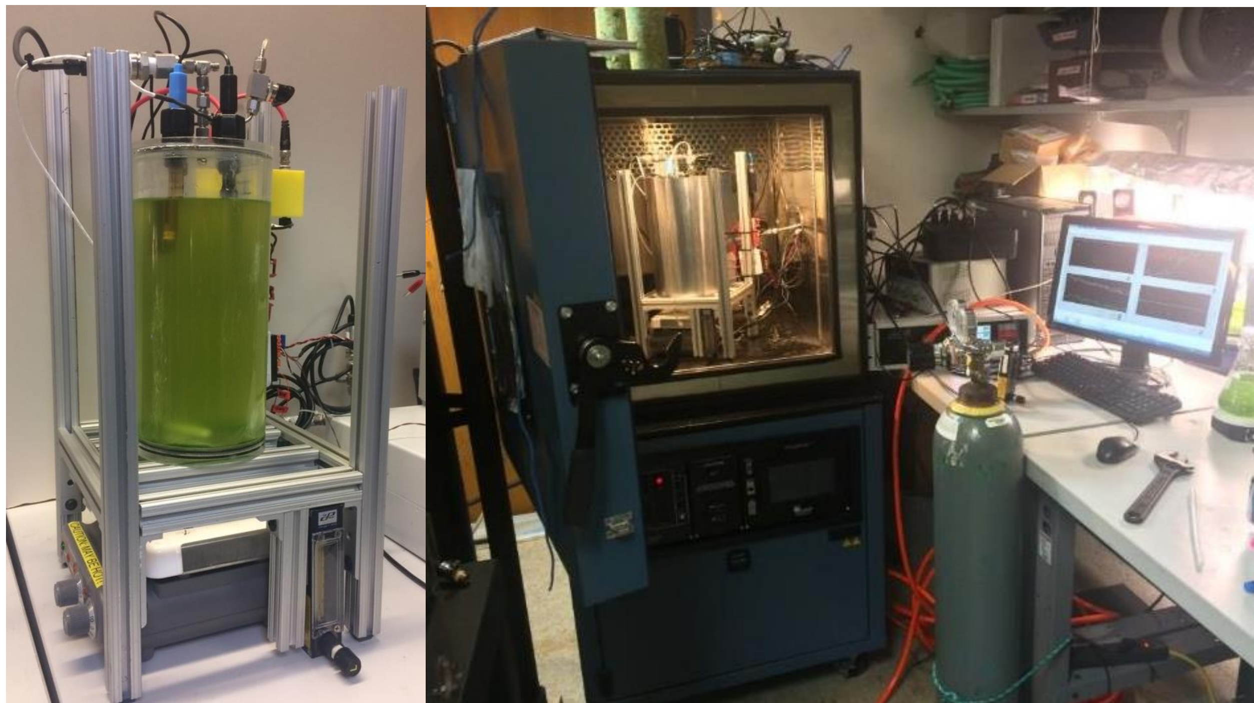

Figure 18: Experimental test setup

The required air composition can either be achieved by a custom premixed gas cylinder or by the use of mass flow controllers mixing individual supply cylinders. Consistent pressure and flow rate are achieved with pressure regulators and rotameters. A check valve together with a ball valve, immediately below the reactor, both prevent the gravity-driven flow of algal solution through the pipes in case the

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air flow is stopped or the photobioreactor gets detached. Besides exchanging gases between the gaseous and the liquid phase, the bubbles also fulfill the purpose of mixing the algal solution within the reactor to achieve a uniform distribution. Furthermore, a magnetic stir bar helps to prevent cells from settling on the bottom of the reactor.

The top of the photobioreactor has an outlet connection that allows the air of the reactor's headspace to escape. Metal tubing guides the air to the bottom of a calcium chloride ($CaCl_2$) desiccant bed. This granulate bed dries the gas stream with a relative humidity content of about 80 % by absorbing water molecules. After leaving the desiccant bed, the gas is pulled out by a diaphragm pump. Here, the chamber pressure is changed from the internal pressure set point (above or below atmospheric pressure) back to atmospheric conditions of about 85 kPa in Boulder. The diaphragm pump is necessary upstream of the gas analyzer, to provide the required ambient conditions at the gas analyzer for precise measurements. The exhaust stream from the gas analyzer is subsequently vented to the ambient environment.

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

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algal photobioreactor with small quantities of growth medium to adjust for the sinking water level due to water loss with the airflow.

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

Table 10: Sensor specifications (g – gaseous phase, l – liquid phase, s – solid)

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

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5.1.2.2 Sensor design

The entire setup is equipped with sensors to measure algal metabolism (CO_2, O_2) and environmental conditions (T, p, pH, DOX, EC). Locations of the sensors are shown in Figure 17 with details of the sensors provided in Table 10. All sensors interface either via analog signal or serial communication to the computer and are received, processed, and displayed via LabVIEW. The program also logs the data in a Technical Data Management Streaming (TDMS) file that can be read by analysis tools such as Microsoft Excel or MATLAB. The program also controls the light cycle of the LED lighting via a digital output and a solid-state relay.

There are 4 probes fed through the top lid of the photobioreactor which can be seen in Figure 18. They measure pH, dissolved oxygen (DOX), conductivity, and temperature directly in the algal solution. The absolute pressure is recorded directly at the outlet of the reactor. Additionally, an absolute pressure sensor is installed at the inlet of the gas analyzer, as the measurements of the gas analyzer are pressure-dependent. The gas analyzer measures carbon dioxide and oxygen concentration through a nondispersive infrared (NDIR) sensor and an electrochemical fuel cell.

5.1.3 Challenges

In a flow-through setup, such as presented in this work, the accuracy and resolution of the sensors plays a critical role in measuring the algal metabolism. Due to the continuous gas flow and limited photobioreactor size, the rate of change in gas composition is smaller than in closed loop systems that accumulate variations over time. Initially, it was thought that the most accurate results would be obtained

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directly in the gas phase of the photobioreactor. However, tests that used small scale sensors showed that a resolution of 1000 ppm gas concentration and uncontrolled effects due to humidity, pressure, and temperature prevent the collection of useable data. The photobioreactor was hence updated to accommodate a gas analyzer with a resolution of 10 ppm. Due to the experimental configuration and size of the analyzer, however, only the exhaust gas from the photobioreactor can be measured. Through the diaphragm pump and gas dryer unit, the gas stream is pretreated to match the gas analyzers narrow input gas parameters in terms of humidity and pressure. The continuous flow also demands low leak rates below $1 \cdot 10^{-4}$ Pa $\cdot m^3/s$ in the system to ensure the precision needed for the required gas measurements and to enable the desired metabolic calculations.

Part of the challenge in achieving high resolution gas measurements is the provision of dry gas to the gas sensors. Drying the air stream is commonly done with silica gel (SiO_2) or Drierite. However, the silica gel also absorbs carbon dioxide, which later gets released again when the desiccant bed becomes saturated with water. As the carbon dioxide produced by the algal cells is measured, this secondary reaction in the desiccant bed falsifies the results. This observation has previously been reported in the research community (Elia et al., 1986). To avoid this problem, calcium chloride ($CaCl_2$) was selected that shows a very low affinity to both carbon dioxide and oxygen. Measurements can also be inadvertently affected due to secondary reactions in the photobioreactor. The solubility of carbon dioxide and oxygen in water is, for example, dependent on the temperature. A temperature fluctuation of less than 1 K therefore

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has to be maintained in the algal solution to adequately minimize temperature dependent transients in dissolved gases.

Cell counts are performed on a small sample and then extrapolated to the entire photobioreactor volume. In order to achieve a representative sample, the algal solution is required to be well mixed without any sedimentation or biofilm formation on the walls. In order to minimize biofilm formation, the surface finish as well as the materials have to be taken into account. Porous surfaces, as found in diffuser stones and certain materials, such as Teflon, attract algal growth on their surfaces and have to be avoided to guarantee a well-mixed culture (Schnurr and Allen, 2015).

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

5.2 Results and discussion

In order to experimentally characterize the testbed physical performance, a photobioreactor filled with distilled water and growth media was run for 40 hours under the desired test conditions. The input gas stream consisted of air enriched to 0.1 % carbon dioxide and was fed into the photobioreactor maintained at a constant

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water temperature of 30°C. This test was conducted under a reactor pressure of 85 kPa. It was verified that the day/night cycle did not induce a secondary effect on the sensor measurements, as can be seen from the constant values in Figure 19 (left). In addition to this control test, a pilot test run with *Chlorella vulgaris* was conducted for 72 hours under otherwise identical conditions. It can be seen that the metabolism changed in response to the light activity cycles over the duration of a day in Figure 19 (right). Comparing the control to the test showed that the measured changes are due to the algae and not due to the altered temperatures which would cause altered solubilities of the gases in the liquid phase. Within an hour of illumination, an increased oxygen evolution can be observed, which is maintained throughout the daylight period. Both the dissolved oxygen as well as the oxygen concentration in the gas stream show this trend. Carbon dioxide levels can be seen to decrease during the day due to increased fixation rates, with pH consequently increasing as result of the reduction in dissolved carbon dioxide. Further tests were conducted for six weeks total duration (three replicates of two different conditions for one week each) in the fall of 2017 that demonstrated the repeatability of the pilot test results obtained in this photobioreactor system.

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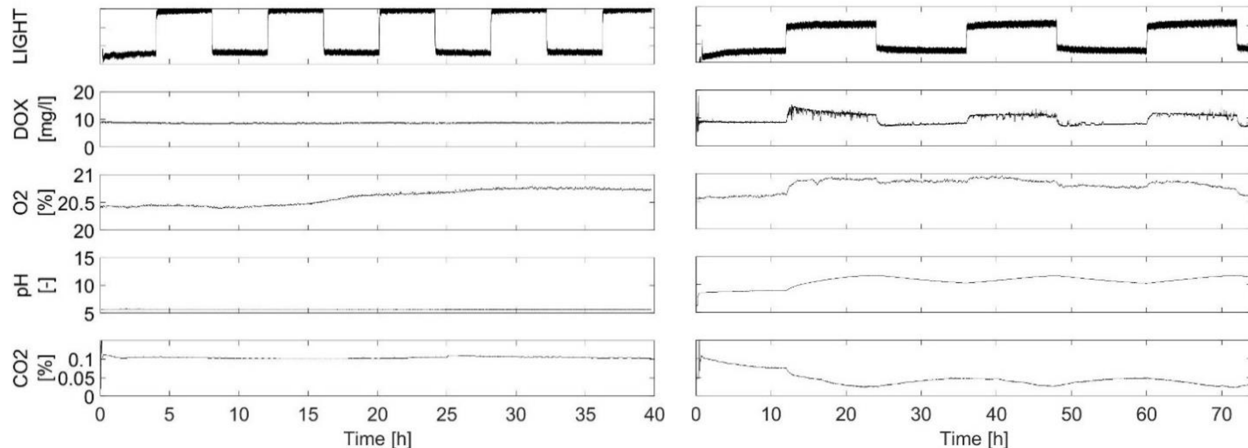

Figure 19: Reference measurements of water and growth media filled photobioreactor without any organisms (left) and measurements of algal filled photobioreactor (right)

The sample port allows for further analysis such as absorptivity measurements using a photometer and cell counts on negligible small samples. Cell absorptions where performed at 450, 600, and 690 nm. A trade between sensitive and robust measurements at wavelengths at the absorption centers (450 and 690 nm) and between them (600 nm) has been previously suggested (Myers et al., 2013). Analysis of the three absorptivities tested in this experiment has shown close correlation between them. Therefore, a sensitive wavelength of 450 nm that closely matches the linearity of the Beer-Lambert law was selected for future experiments (Myers et al., 2013). Figure 20 shows the measured growth curve of *Chlorella vulgaris* in the newly developed photobioreactor. It resembles a typical algal growth curve consisting of a lag phase during the first day, followed by an exponential phase from day 1 until day 5 and, finally, transitioning to a saturation phase from day 5 on. The cell density at the start was $1 \cdot 10^6$ cells/ml and increased to a maximum of $1 \cdot 10^8$ cells/ml with a typical cell viability above 95 %. This compares to a maximum reported cell concentration of $1 \cdot 10^9$ cells/ml under optimized conditions (Lee and Palsson, 1995).

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During the maximum growth rate, a doubling time of 20.9 hours was achieved. This compares to a maximum reported doubling time of 9 hours under optimized conditions (Maxwell et al., 1994). The pH of the algal solution stayed relatively constant, between 9 and 11, during the entire test duration without active control. Salinity, pressure, and water temperature measurements were used as verification of sufficient nutrient availability as well as constant pressure and water temperature during the experiment.

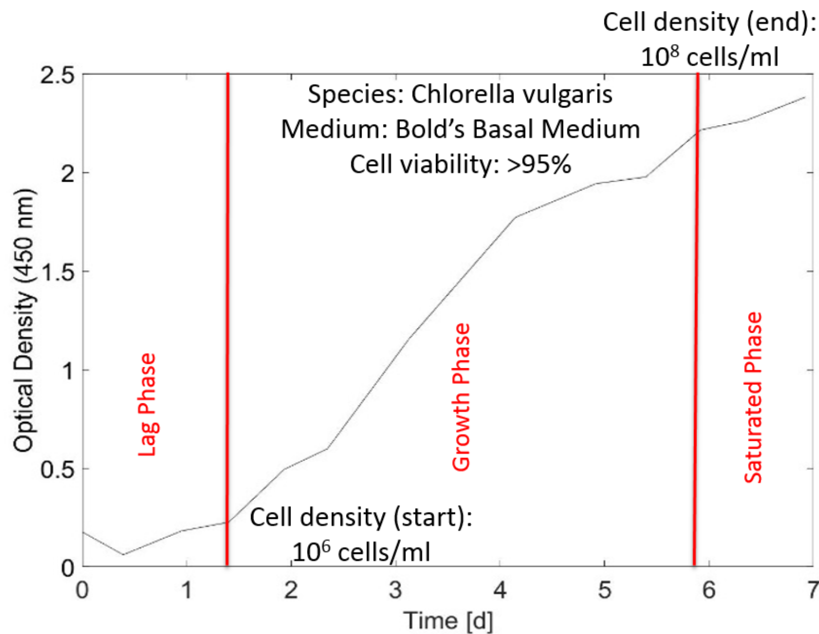

Figure 20: Growth curve of Chlorella vulgaris cultivation in photobioreactor for 7 days at 450 nm

Different environmental conditions, such as pressure, will affect the algal metabolism. The output carbon dioxide and oxygen concentration, the cell densities, and the growth rate was used in later studies to characterize the effect from a system perspective. To measure the carbon dioxide fixation and oxygen evolution rate, recorded output carbon dioxide and oxygen concentrations are subtracted from the known input concentrations. The integrated values for total oxygen evolution and

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carbon dioxide concentration are then compared to the absolute cell number increase for establishing a relationship between algal metabolism and biomass production.

5.3 Conclusion

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

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implement an algal photobioreactor in a spacecraft. Even though primarily intended for conducting studies under reduced pressure, this setup can also be used to evaluate algae cultures under a variety of other conditions of interest for spaceflight applications, as well as for related terrestrial purposes.

5.4 Resultant publications and presentations

Conference Papers (refereed)

1. **T. Niederwieser**, R. Wall, J. Nabity, and D. Klaus, "Development of a testbed for flow-through measurements of algal metabolism under altered pressure for bioregenerative life support applications;" in 47th Int. Conf. Environ. Syst. Charleston, SC, 2017 (ICES-2017-23)

Presentations

1. 47th International Conference on Environmental Systems (ICES), "Development of a testbed for flow-through measurements of algal metabolism under altered pressure for bioregenerative life support applications" Charleston, SC, 2017.

5.5 Resultant outreach component

Undergraduate Involvement

1. University of Colorado Boulder, Undergraduate Research Opportunities Program (UROP), Ryan Wall, May 2016 to August 2017

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6 The effect of altered nitrogen partial pressure on the performance of Chlorellaceae for spacecraft applications

6.1 Introduction

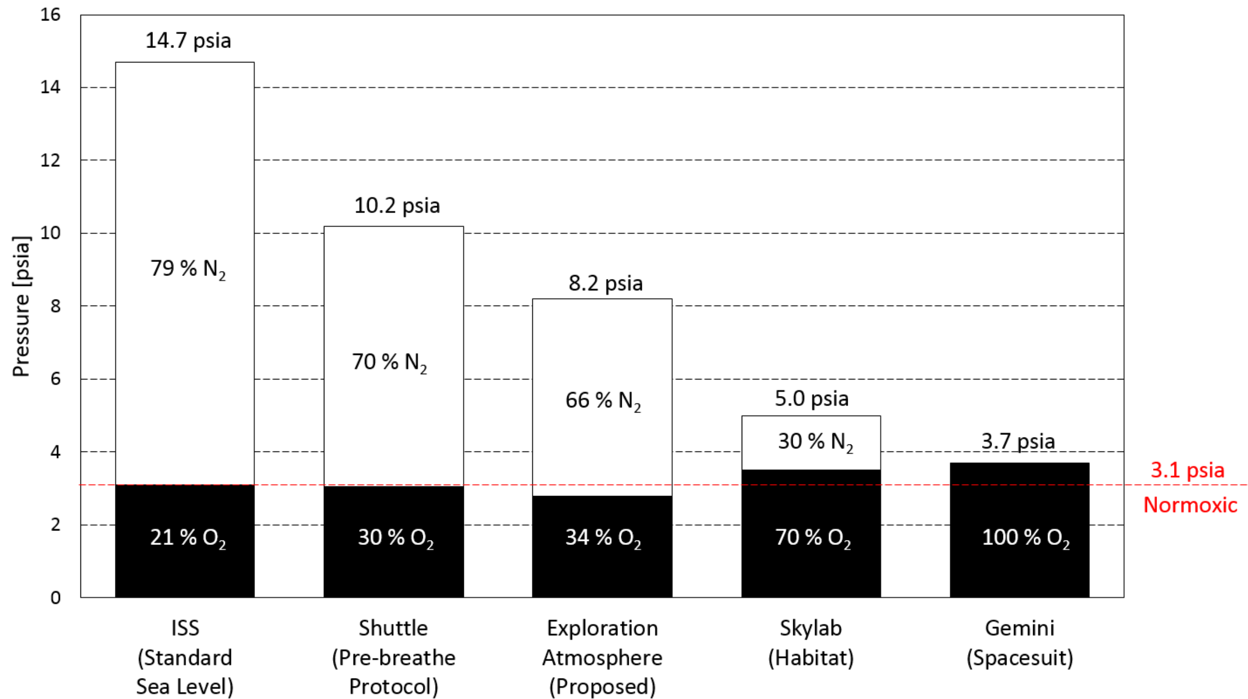

Figure 21. Comparison of standard sea level atmosphere with NASA's proposed exploration atmosphere

It is highly likely that algae in a life support system will be exposed to the same cabin atmospheres as the crew. Spacecraft cabin atmospheres are influenced by the required human physiological oxygen partial pressure, flammability concerns, as well as the risk of decompression sickness during transitions to EVA's. Flown implementations have included, but are not limited to, 3.7 psia / 100 % O2 (early U.S. Spacesuit), 5 psia / 70% O2 (Skylab), 10.2 psia / 30 % O2 (Shuttle pre-breathing) as well as standard sea level conditions of 14.7 psia / 21 % O2 (ISS). Most recently, NASA

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has proposed the "Exploration Atmosphere" consisting of 8.2 psia with 34 % oxygen (see Figure 21). This composition is a compromise between an acceptable risk of decompression sickness during transition into the spacesuit and risk of flammability (Norcross et al., 2013, 2015). The Exploration Atmosphere will be especially crucial in surface habitats that require Extravehicular Activities (EVA) at a high frequency of approximately once per day as no pre-breathing will be necessary.

6.2 Background

Even though a reduced pressure of 8.2 psia can be found on Earth at an elevation of approximately 4500 m, the oxygen partial pressure diminishes with altitude as Earth atmosphere features a constant oxygen concentration of 21%. The exploration atmosphere conditions hence represent a very unique condition not researched by terrestrial biologists before. In consequence, there are only very limited published data on the effect of pressure on the growth of algae. Qin et al. (2014) have looked at the effect of reduced pressure on cyanobacteria while maintaining the same environmental concentrations. It was shown that half the environmental pressure causes about a 50 % reduction in growth rate (Qin et al., 2014b). This behavior suggests that the reduction in pressure leads to the same reduction in partial pressure of carbon dioxide and hence total amount of carbon dioxide available to the algae. Additionally, for sampling purposes, the cyanobacteria were exposed to pressure swings every second day to atmospheric conditions that most likely created additional stress within the algae. The applicability of the aforementioned study to bioregenerative life support systems is very limited, as it focused exclusively on

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cyanobacteria and did not include any green algal species. Furthermore, the reduced oxygen partial pressures in these experiments is unlikely to be used in a crewed space habitat due to human physiology constraints.

At the other hand, Orcutt et al. have shown that the alga *Chlorella sorokiniana* grows faster at reduced pressures in helium – carbon dioxide mixtures when the carbon dioxide partial pressure is kept constant at 0.735 psia (which correlates to about 5 % concentration at standard sea level pressure) (Orcutt et al., 1970). For example, a pressure of 3.5 psia resulted in an almost 30 % increase in growth rate. A subsequent experiment that exchanged the diluent helium gas with oxygen at the low-pressure showed no effect between the two different diluent gases. This work demonstrated that growth of *C. sorokiniana* is unaffected by the diluent gas at those low pressures. Unfortunately, no metabolic measurements were performed, and the culture apparatus was not explained in great depth to replicate the study.

Other studies have focused exclusively on increased atmospheric pressures (Hannan, 1964; Richardson et al., 1969). It was shown that both increased atmospheric pressures as well as increased oxygen partial pressures lead to slight reductions in growth rates. Low pressure experiments with algal cultures in Mars regolith simulant in pure carbon dioxide environments were also performed between 50 and 1000 mbar. Most interesting, green algae and cyanobacteria showed different behaviors, with the green algae performing best under a carbon dioxide partial pressure of 300 mbar (which correlates to about 30 % concentration at standard sea level pressure) (Thomas et al., 2008). However, at standard sea level pressure, the

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optimum carbon dioxide concentration was experimentally shown to be around 5 % (Bhola et al., 2011; Chinnasamy et al., 2009; De Morais and Costa, 2007; Singh and Singh, 2014).

It should be noted that the investigated pressures are all relevant for the internal pressure vessels of a space habitat. Lower vacuum pressures as exist in the space environment are not looked at in this study as there is not sufficient carbon dioxide for algal respiration. Nevertheless, it has been shown that dormant and dehydrated algae are able to survive the space environment (temperature, pressure, radiation) for a minimum of 18 months as the majority of the samples developed into new cultures in laboratories upon return to Earth (Baqué et al., 2017; Cockell et al., 2011).

Algal growth under the exploration atmosphere has not been characterized before, but this is a crucial step to develop future algal life support systems. Data from past studies showing increased growth at lower pressures justify further research that could potentially lead to more efficient algal systems. Especially flow-through measurements of metabolic parameters in algal photobioreactors under continuous optimum conditions (vs. under deteriorating batch conditions) are detrimental for future simulations of air revitalization technology performance. One potential hypothesis is that at lower pressures an increased diffusion rate of both carbon dioxide and oxygen in nitrogen could result in increased growth of algae. The diffusion coefficient is dependent on temperature and pressure as shown in Equation

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4 (R. Welty et al., 2008). For pressures between 0 and 367 psia, the diffusion coefficient varies inversely with pressure.

$$D_{AB_{T_2,P_2}} = D_{AB_{T_1,P_1}} \left(\frac{P_1}{P_2}\right)\left(\frac{T_2}{T_1}\right)^{\frac{3}{2}} \frac{\Omega_{D|T_1}}{\Omega_{D|T_2}} \quad (4)$$

At low environmental pressures, achieved solely through reduction of nitrogen partial pressure, the increased diffusivity could be used to enhance productivity of carbon dioxide-limited photobioreactors. Compared to standard sea level composition (14.7 psia), the exploration atmosphere (8.2 psia) could increase performance by 79%, a result that has to be experimentally verified.

6.3 Materials and methods

6.3.1 Strain and stock culture conditions

Chlorella vulgaris Beyerinck (Carolina Biological Supplies, *Chlorella vulgaris*, strain 152075) was chosen due to its fast growth rate, fully edible biomass, high volume efficiency, ease of culturing, and being well documented in the literature (Barghbani et al., 2012; Daliry et al., 2017; De Morais and Costa, 2007; Lee et al., 2001). Algae were cultured in Bold's Basal Medium (BBM) (PhytoTechnology Laboratories, BBM Solution 50x, B1650) diluted in Ultrapure water as a compromise between high growth rates and commonly used growth media in previous published literature (Sharma et al., 2011). Algae were diluted in culture with a factor between 1:15 and 1:30 to achieve a cell population density of 1*10^6 cells/ml and the concentrated growth media was diluted in ultrapure water with a factor of 1:50. One batch of stock culture was kept in the lab and samples of that culture were used as

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the inoculum source for all specified experiments. The culture was kept at room temperature in a covered 2-liter beaker with a magnetic stir bar and active airflow over the top of the surface of the water. A LED light bulb (Earthbulb, A19 OMNI LED) with 810 lm and a color temperature of 5000 K, set to a 12:12 cycle, was the light source delivering 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ within the PAR range to the surface of the reactor. At the time of the first experiment, the strain was cultured under these same conditions for more than two years, so that the alga was acclimated to the lab conditions. The culture conditions were non-axenic but clean, using only autoclaved or disposable sterile products in an open lab environment. Culture conditions during the actual experiment are specified in detail in section 6.3.3.

6.3.2 Experiment design

6.3.2.1 Variables and constants

As the independent variable in the experiment, the total pressure inside the reactor is achieved solely through a change of the nitrogen partial pressure with constant oxygen and carbon dioxide partial pressure. Dependent variables were measured in three locations: First, dissolved oxygen and pH inside the liquid phase of the reactor; second, the concentration of carbon dioxide and oxygen in the gaseous phase coming out of the reactor; and, lastly, the optical density and cell population density measurements performed daily on 1 ml algal culture samples. Constants within the reactor were temperature, partial pressure of oxygen and carbon dioxide going into the reactor together with their respective mass flow (Table 11), light cycle, light intensity, as well as the inoculum characteristics (cell population density,

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growth media concentration). Potential confounding variables, such as the shadowing of the cells within the reactor as they grow and the total gas flow through the reactor (as a result of the changing nitrogen flow) were identified.

6.3.2.2 Conditions

Table 11: List of experiment conditions with their respective gas characteristics

Name	Total Pressure [psia]	Total Flow [slpm]	CO2			O2			N2		
			[%]	[psia]	[slpm]	[%]	[psia]	[slpm]	[%]	[psia]	[slpm]
Exploration Atmosphere	8.2	0.8	1.8	0.147	0.015	38	3.1	0.288	60.2	4.94	0.482
Standard Sea Level	14.7	1.37	1.0	0.147	0.015	21	3.1	0.288	78.0	11.47	1.069

The gas compositions were achieved by using pure gas cylinders (nitrogen >99.999 %, oxygen >99.5 %, carbon dioxide >99.5 %) as well as mass flow controllers. The partial pressure for carbon dioxide and oxygen was kept constant by providing the same mass flow (± 0.2 % of set value) of those two gases into the system in all cases. The only parameter that changed was the mass flow of nitrogen which changed the partial pressure of nitrogen in the system. Due to Daltons Law, the total pressure in the system changed accordingly.

6.3.2.3 Test plan

The experiment contains two conditions as described in section 6.3.2.2 as well as three replicates for each condition resulting in a total of 2x3=6 experiment runs. Each experiment was run for a total duration of one week leading to a total experiment duration of 6x1=6 weeks. The duration of one week was experimentally chosen in pilot studies to record both the adaptation phase to the reactor (3 – 4 days)

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as well as a stationary phase (3 – 4 days). In addition to a thorough characterization of the testbed as published earlier (Niederwieser et al., 2017), a one-day leak test as well as a one-day steady flow test was performed with the completely assembled test setup just before the actual experiments.

6.3.3 Experimental setup

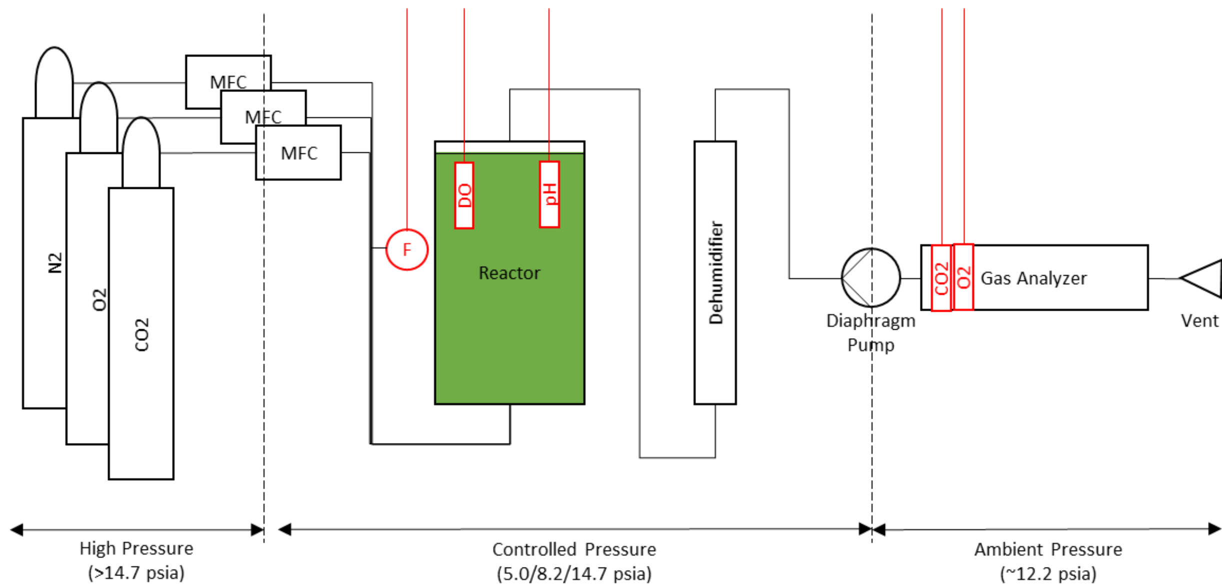

Figure 22: Piping and Instrumentation Diagram of the test setup

The experiment setup follows a flow-through approach to guarantee constant culture conditions during the experiment and measure the metabolic rates of the algae. As seen in Figure 22, the setup can be divided in three different parts characterized by their internal pressure. Starting on the left is the gas supply part consisting of three gas cylinders containing oxygen, nitrogen, and carbon dioxide as well as one mass flow controller (MKS Instruments, Inc., GE50A) for each of the three gases. The middle section contains the actual photobioreactor which can be seen in Figure 23. Between the mass flow controllers and the diaphragm vacuum pump

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(GAST, DOA-V110-FB), the pressure can be controlled in the reactor between 5 and 14.7 psia. The reactor itself is a custom-made acrylic cylinder with an internal volume of 3 liters. The gas stream is sparged through the reactor from the bottom. To assure a well-mixed culture both for growth as well as for sampling purposes, a magnetic stir bar is included in the reactor bottom in addition to the sparging induced mixing. The reactor is surrounded by a shroud that holds LED lighting strips (Home EVER Inc., 4100057-DW) and also acts as an aluminum heat exchanger to air for the thermal heat from the lighting. The vacuum pump pulls the air from the headspace through an opening in the top of the reactor into a calcium chloride (HomeScienceTools, CH-CACL500) bed acting as dehumidifier (Drierite, Gas Drying Unit, 26840). The entire section between mass flow controller and diaphragm pump is contained within an environmental chamber. The dry airstream is then fed into the gas analyzer (CAI Instruments, ZRE Gas Analyzer) for carbon dioxide and oxygen concentration measurements before being vented into the lab environment. A more detailed description of the test setup together with characterization tests was published earlier (Niederwieser et al., 2017).

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Figure 23: Part of the actual test setup that is contained in the environmental chamber

6.3.4 Real-time measurements

The test setup features a variety of continuous measurements at a sampling rate of 1 Hz during the entire duration of the experiment. Several probes measure the pH (Atlas Scientific, pH Kit), dissolved oxygen (Atlas Scientific, Dissolved Oxygen Kit), Conductivity (Atlas Scientific, Conductivity Kit K 0.1), and water temperature (Atlas Scientific, PT-1000 Temperature Kit) within the liquid in the culture reactor. Additionally, carbon dioxide and oxygen concentration were measured on the outlet of the reactor using a gas analyzer (California Analytical Instruments, ZRE Gas Analyzer). Pressure measurements were conducted in the reactor head space, as well

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as in the inlet and outlet of the gas analyzer using pressure transducers (Omega Engineering, Pressure Transducer, PX309-030A5V).

Data recordings were performed using LabVIEW (National Instruments, LabVIEW 2013). Analog signals from the pressure sensors and thermistors were fed into a DAQ (National Instruments, USB6009). Digital outputs of the same device control both the timed light cycle as well as a logic-controlled miniature valve (Clippard Instrument Laboratory, Inc., EV-2-24) that controls the pressure within the reactor to ±2 %. Digital signals from the DO, pH, conductivity, and temperature probes within the reactor were interfaced via USB using a UART protocol. Lastly, the gas analyzer data was recorded using a MODBUS digital protocol via USB. The LabVIEW code records all data in a single tdms data file.

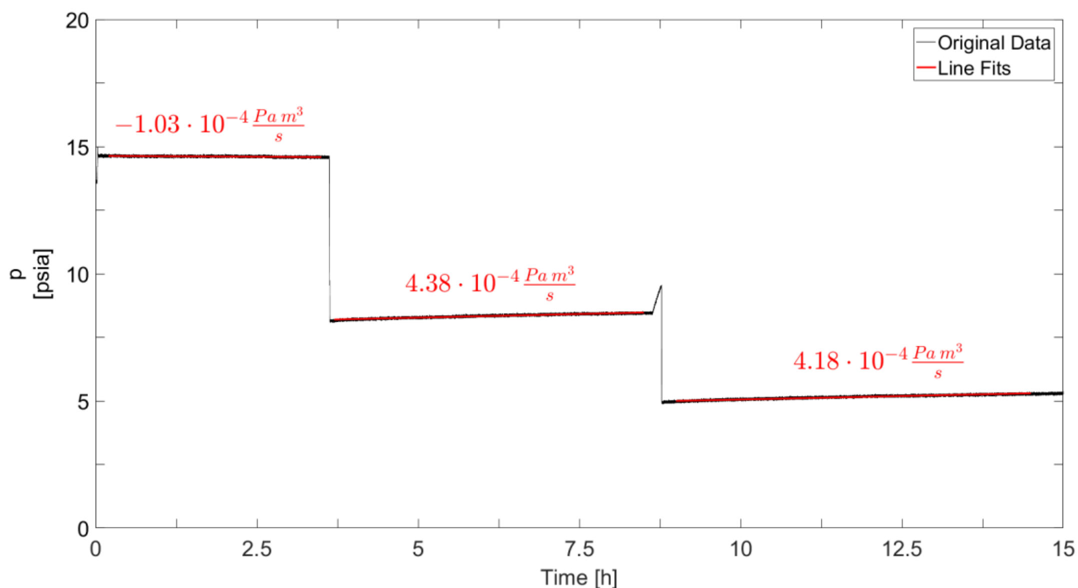

Figure 24: Leak characterization tests at 3 different conditions for a minimum of 3 hours each. Note that the atmospheric pressure for Boulder is about 12.2 psia.

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Since this experiment was conducted in Boulder, Colorado, with an atmospheric pressure of about 12.2 psia, the different leak directions in and out of the reactor can influence the experimental data. It was therefore of crucial importance to minimize the leak rate of the system and characterize it. In order to obtain the leak rate, the system was consecutively conditioned to the three different pressures and sealed off at the inlet and outlet with manual valves and leak rates of $-1.03 \cdot 10^{-4}$, $+4.38 \cdot 10^{-4}$, and $+4.18 \cdot 10^{-4}\ \frac{Pa \cdot m^3}{s}$ were graphically obtained using a linear fit and the internal volume of the system of 0.004 m^3 for the three different cases of 14.7, 8.2, and 5 psia (see Figure 24). To find out the mass flow rate and compare it to the nominal gas flow Equation 5 was used (Rottländer et al., 2016).

$$\frac{\Delta m}{\Delta t} = \frac{q_L M}{RT} \tag{5}$$

Using the universal gas constant R (8.314 $\frac{J}{mol \cdot K}$), a temperature of 30°C (303.15 K), as well as the molar mass of air M (29 $\frac{g}{mol}$), the observed leak rates result in a mass flow of $-1.19 \cdot 10^{-7}$, $+5.04 \cdot 10^{-7}$, and $+4.81 \cdot 10^{-7}\ \frac{g}{s}$ for the three different pressures of 14.7, 8.2, and 5 psia. This is in contrast to the gas flow of 0.028 and 0.018 $\frac{g}{s}$ for the two different experiment conditions of 14.7 and 8.2 psia and therefore represents errors of 0.0004 and 0.003 %. This error is smaller than the measurement accuracy of the sensors and is therefore negligible.

Additionally, the effect of hydrostatic pressure due to the water column inside the reactor was calculated to be 0.36 psia at the bottom of the reactor (Equation 6).

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Compared with the two reactor pressures of 8.2 and 14.7 psia, the constant error is 4.3 and 2.4 % and therefore determined small enough to be neglected.

$$p_{hydrostatic} = \rho\ g\ h = 1000\frac{kg}{m^3} \cdot 9.81\frac{m}{s^2} \cdot\ 0.25\ m = 2453Pa = 0.36\ psia \qquad (6)$$

6.3.5 Cell counts and photometer readings

Optical density was measured daily using a photometer (Thermo Scientific, Multiskan FC) at a wavelength of 450 nm. Each measurement was done in triplicate in a 96-well plate filled with 200 µl in each well. Before each measurement, the sample was shaken at the slow setting for 2 seconds to prevent the algae from settling.

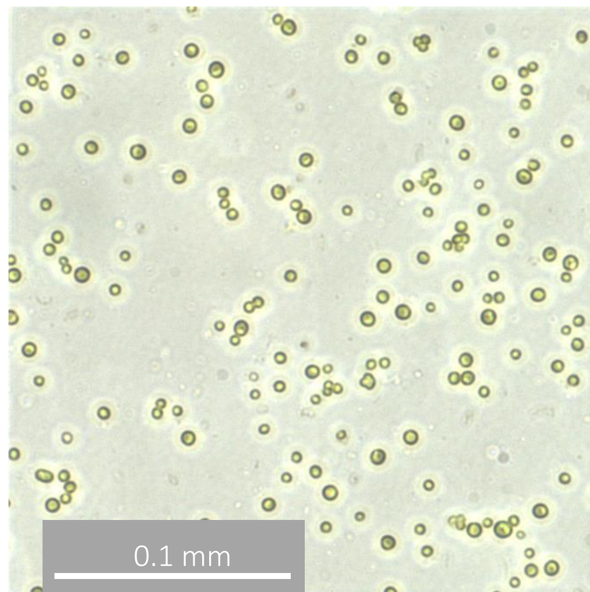

Figure 25: Microscopic image of the culture

Cell counts were performed daily using a hemocytometer (Hausser Scientific, Reichert Bright-Line, 1940). For the cell counts, samples were diluted with ultrapure water to achieve a cell density of approximately 100 per square millimeter. Five squares were counted and averaged to calculate a reliable cell population density

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according to the manufacturer's protocol (Hausser Scientific, 2018). Cell viability measurements using dye (Sigma-Aldrich, Trypan Blue Solution, T8154) were not performed with the daily checks, as pilot studies at different pressures did show no detectable changes at cell viability levels of about 95 %.

6.3.6 Metagenomic sequencing

Both at the beginning and at the end of each experiment run, 3 ml samples of the algal culture were taken out of the photobioreactor. The samples were centrifuged and diluted with ultrapure water to concentrate the biomass and subsequently stored in a freezer at -80 °C in RNAlater. Three of those samples (Run 1, 3, and 6) taken at the end of each run were used to perform DNA extraction, library preparation (350 bp insert DNA library), and metagenomic analysis (Illumina Platform PE150, Q30≥80%) with a resolution of 3 Gb of raw data per sample (Novogene Corporation, Inc., Chula Vista, CA). For the analysis, filtered reads were first assembled to generate scaftigs before genes were predicted using MetaGeneMark. After dereplication, all of the unique genes were used to construct a gene catalogue. The host sequence of *Chlorella vulgaris* was then removed from the catalogue before the annotation. For taxonomy annotation, BLAST was performed against the MicroNR database (NCBI: version 2016-11-05) to generate annotation information of gene catalogue. For function annotation, BLAST searches were performed against the 3 databases KEGG, eggNOG and CAZy. For whole genome mapping, 5,000 random reads per sample were selected and compared with whole genome database (NCBI: version 2016-11-05).

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6.3.7 Data processing

Continuous data recorded in the tdms file were loaded into MATLAB (MathWorks, Inc., MATLAB R2018a) for data processing. Data were filtered from noise and occasional spikes using the built-in medfilt1 function. Averages of the steady states were taken for each measurement (cO2, cCO2, pH, DO) during the last three days separated for day and night as indicated by the green and red intervals shown in Figure 26 and Figure 27. The average was taken for only 8 hours out of each 12-hour cycle to avoid the dynamic behavior resulting from the light switching. Occasionally, intervals were manually excluded due to obvious offsets or temporary sensor malfunctions (bubble interference at the probe, communication loss, etc.), reducing the number of intervals to two full days. Using the oxygen and carbon dioxide concentration as well as the flow rate, the oxygen evolution and carbon dioxide fixation was calculated to have a measurement that can be easily compared between the different conditions. Likewise, the DO and pH values between day and night were subtracted from each other to gain more meaningful ΔpH and ΔDO values. The dissolved oxygen sensor changes its calibration due to the sudden pressure changes affecting the electrolyte solution inside the probe. This resulted in either high or low readings based on the electrolyte refill level before each test and the amount of leaked electrolyte through the membrane during the sudden pressure change. The gain was normalized to the calculated 8.2 point in post-processing. The sensor offset remained unchanged.

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In runs 3 and 4, the pH sensor stopped operating which resulted in a loss of data for the pH channel. As those two instances happened once in each the 8.2 and the 14.7 psia case, the data were removed in those two runs and analysis was done with a sample size of 2 instead of 3 for pH.

Data from the gas analyzer for the oxygen values in run 3 were unreliable and could not be explained. A hypothesized leak could be excluded through leak testing as well as the fact that the carbon dioxide values still showed the expected behavior. Data from the two erroneous runs were excluded and the analysis was done with a sample size of 2 instead of 3 for gaseous oxygen.

For statistical reliability experiments were performed in triplicate. cO2, cCO2, pH, DO, cell population density and optical density data from all replicates were averaged for both conditions and the standard deviation was calculated. Student t-test was performed using Microsoft Excel software to determine the statistical significance.

6.4 Results and discussion

6.4.1 Raw data comparison

In Figure 26 and Figure 27, two exemplary raw data plots can be seen for both the 8.2 and the 14.7 psia cases. The adaptation phase during the first 3 – 4 days can be seen as well as the stationary phase during day 5 – 7. Another clear observation is the diurnal cycle caused by the 12:12 light cycle.

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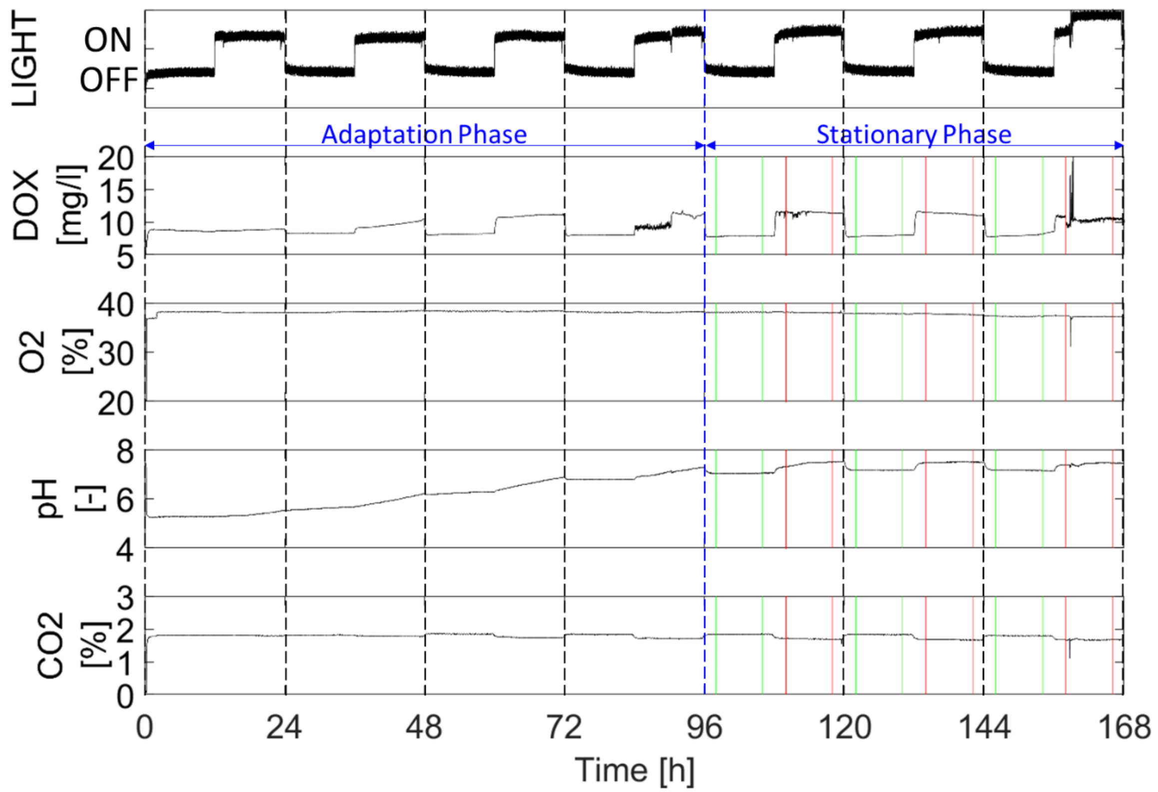

Figure 26: Exemplary experiment run at 8.2 psia

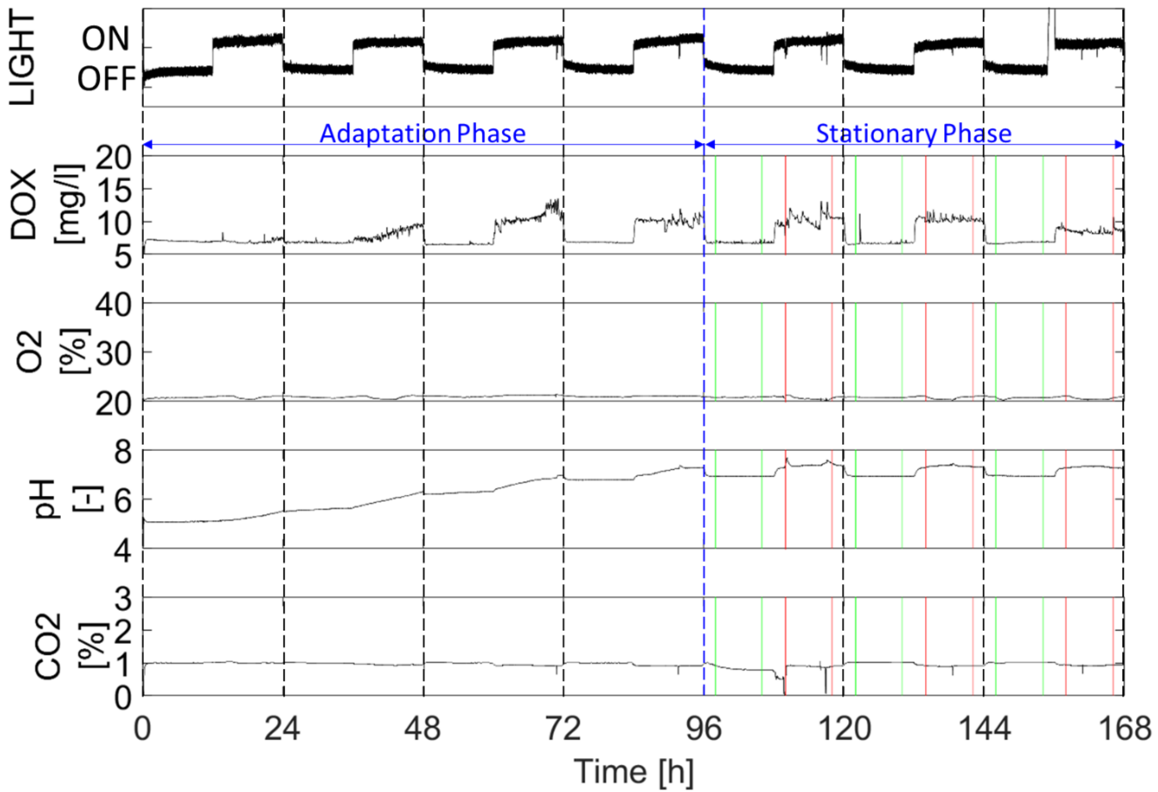

Figure 27: Exemplary experiment run at 14.7 psia

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As expected, algal photosynthesis caused higher dissolved oxygen concentrations in the culture during the day, together with higher gaseous oxygen concentrations on the outlet. This is coupled with higher pH values (lower dissolved carbon dioxide) together with lower gaseous carbon dioxide concentrations during the day. Average values taken from highlighted day (red) and night (green) sections can be seen in Table 12 for all three replicates.

Table 12: Comparison of raw and predicted values during day and night

	Flow [slpm]	DO [mg/l]		pH [-]		CO2 [%]		O2 [%]		PQ [-]
		Night	Day	Night	Day	Night	Day	Night	Day	
8.2 psia										
Run 1	0.8	8.2[1]	11.80	7.11	7.44	1.82	1.68	37.72	37.79	2.1
Run 2	0.8	8.2[1]	11.30	N/A[3]	N/A[3]	1.85	1.70	37.99	38.15	0.9
Run 3	0.8	8.2[1]	13.84	6.64	6.85	1.84	1.69	N/A[2]	N/A[2]	N/A
Average	0.8	8.2	12.31	6.88	7.14	1.84	1.69	37.85	37.97	1.3
Predicted	0.8	8.2		6.93*		1.8		38		
14.7 psia										
Run 1	1.37	8.2[1]	12.54	7.14	7.56	1.00	0.91	20.61	20.68	1.4
Run 2	1.37	8.2[1]	11.95	N/A[3]	N/A[3]	1.00	0.92	20.81	20.96	0.6
Run 3	1.37	8.2[1]	12.54	6.929	7.35	1.00	0.91	N/A[2]	N/A[2]	N/A
Average	1.37	8.2	12.34	7.04	7.45	1.00	0.91	20.71	20.82	0.8
Predicted	1.37	8.2		6.93*		1.0		21		

*using an alkalinity of 120 mg/l.
[1] Corrected for altering calibration as outlined in chapter 6.3.7.
[2] Oxygen sensor mismatch as outlined in chapter 6.3.7.
[3] No obtained data due to dead sensor as outlined in 6.3.7.

The gas concentrations for carbon dioxide and oxygen were established as described in Chapter 6.3.2.2 without further calculations, as it was experimentally demonstrated to be independent of flow rate and pressure in the observed range. During the night, where no photosynthesis is conducted in the cell, the preset gas concentrations of 38 and 21% oxygen as well as 1.8 and 1.0% carbon dioxide for the conditions of 8.2 and 14.7 psia match closely with the measured values in the gas

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analyzer. The photosynthetic quotient was calculated as the ratio between the volume of oxygen released and the volume of carbon dioxide absorbed using Equation 7.

$$PQ = \frac{V_{O_2}}{V_{CO_2}} \tag{7}$$

The calculated PQ falls in the range of previously published data for green algae, but does show significant fluctuations without any consistent trend. The reason for this behavior is unknown. The expected dissolved oxygen content in the culture was calculated using Henry's law (Equation 8), using the observed partial pressure of oxygen in the gaseous phase.

$$c_{DO} = pp_{CO_2} \cdot H(30) \cdot M_{O_2} \tag{8}$$

Henry's constants at the culture temperature of 30 °C needed for implementation in Henry's law were analytically calculated based on Equation 9, given by Sander (2015) (Sander, 2015).

$$H(T) = H^{\theta} \cdot \exp\left(\frac{-\Delta_{sol}H}{R}\left(\frac{1}{T} - \frac{1}{T^{\theta}}\right)\right) \tag{9}$$

For carbon dioxide, the widely distributed and reviewed (Orr et al., 2015) CO2SYS code was used in MATLAB. The pH content was calculated using the atmospheric carbon dioxide partial pressure as well as the alkalinity of the culture. Total pH scale was used as well as a constant alkalinity value of 120 mg/l (experimentally determined in the batch cultures as well as the experiment runs) (Lewis and Wallace, 1998). Dissociation constants used are from Millero for the use with pure water with a salinity of 0 (vs. most constants that are established for seawater) (Millero, 1979). As seen in Table 12, the predicted pH of 6.93 matches in

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both the 8.2 and 14.7 psia case the measured values and therefore indicates the availability of equal absolute amounts of carbon dioxide in the liquid phase.

6.4.2 Effects on growth rate

As shown in Figure 28, the growth rate follows the typical pattern for algae featuring an initial lag phase (days 1 – 2), followed by an exponential phase (days 2 – 6), and transferring over into an onset of the stationary phase (day 7). It can be seen that the dynamic behavior between the two plots is in agreement. Between the two pressure conditions, no apparent differences can be seen.

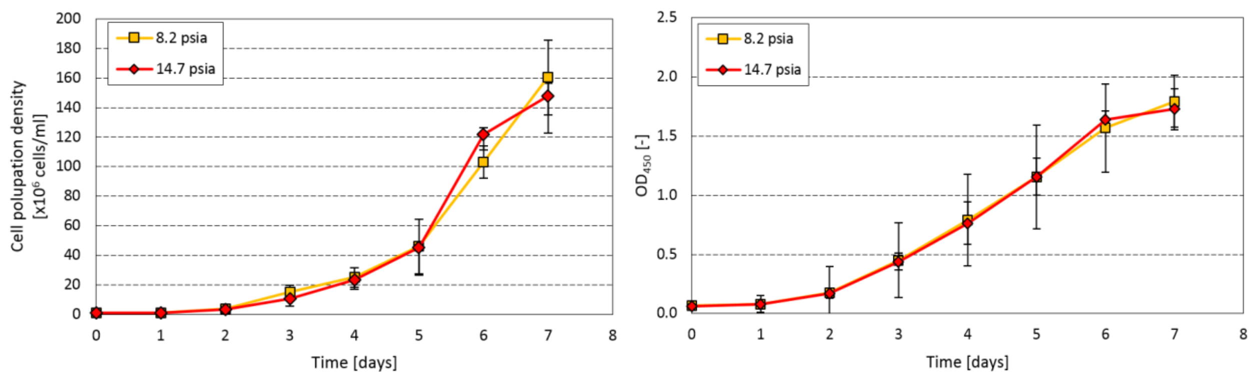

Figure 28: Cell population density (left) and optical density (right) over time for the 2 conditions at 8.2 and 14.7 psia. Error bars represent one standard deviation from the three experimental triplicates.

Comparing the cell population density with the OD_{450} shows good correlation between the two different cases (Figure 29). Both correlations follow the typical path for cell culture studies, which consists of a proportional correlation until the critical OD (~1.0), after which the OD deviates from proportionality (Myers et al., 2013; Widdel, 2010).

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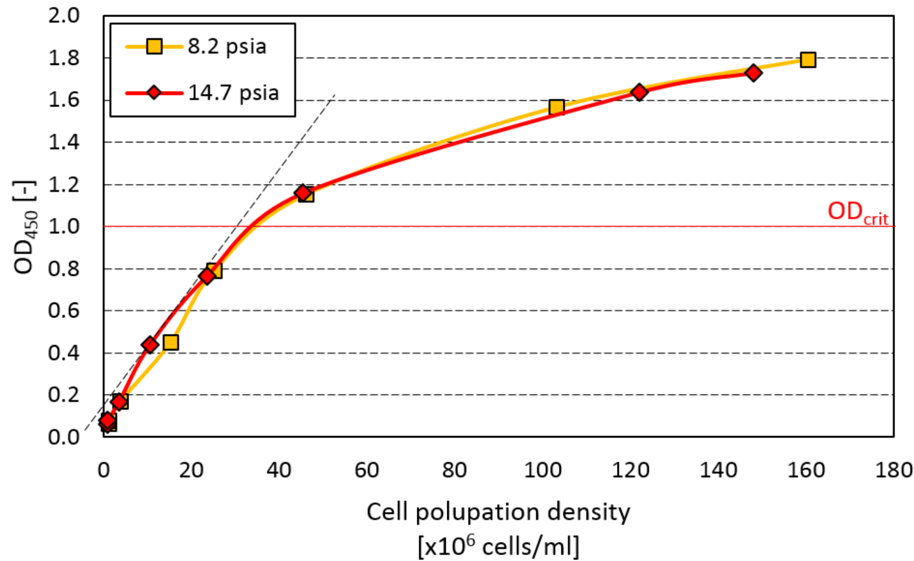

Figure 29: Correlation between cell population density and optical density for the 2 conditions at 8.2 and 14.7 psia. The linear correlation at low concentrations is shown with the black dotted line.

Accordingly, the growth characteristics derived from the cell population density measurements are also very comparable (Table 13). Even though the 8.2 psia conditions favor a slightly slower growth, the differences are not significant within a 95 % confidence interval. A p-value of 0.11 across the three replicates in the two different pressure conditions was calculated using the student's t-test in Excel (Equal sample size, equal variances, 2 samples, 2 tails).

Table 13: Derived growth characteristics (population growth rate, doublings per day, and doubling time) between the two conditions

	r Population growth rate [day^{-1}]	k Doublings [day^{-1}]	T_2 Doubling time [days]
8.2 psia	0.748	1.078	0.927
14.7 psia	0.821	1.184	0.845

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6.4.3 Effects on metabolism

Carbon dioxide fixation and oxygen evolution were continuously measured in the gaseous phase during the illuminated periods of the experiment. Similarly, the change in pH and DO between day and night were measured. Replicate experimental values were averaged for each pressure case and plotted in Figure 30. It can be seen that for carbon dioxide as well as dissolved oxygen there is minimal difference between the two different pressures. A student's t-test (Equal sample size, equal variances, 2 samples, 2 tails) yielded p-values of 0.096, 0.499, 0.972, and 0.140 for CO_2, O_2, DO and pH, respectively. Even though there is a trend across all 4 measurements of reduced metabolism in the 8.2 psia case, the p-values of > 0.05 indicate that we cannot reject the null hypothesis; there is no significant difference (95 % confidence interval) in metabolism between standard sea level atmosphere and the exploration atmosphere. This is in alignment with the identical growth conditions. Comparing the carbon dioxide with oxygen rates it can be seen that oxygen shows bigger differences between the conditions as well as more variability in error bars. This is due to the fact that the oxygen sensor has an order of magnitude less resolution than the carbon dioxide sensor. Additionally, carbon dioxide measurements showed the best reliability during the experiments and is probably the most reliable metric in this study.

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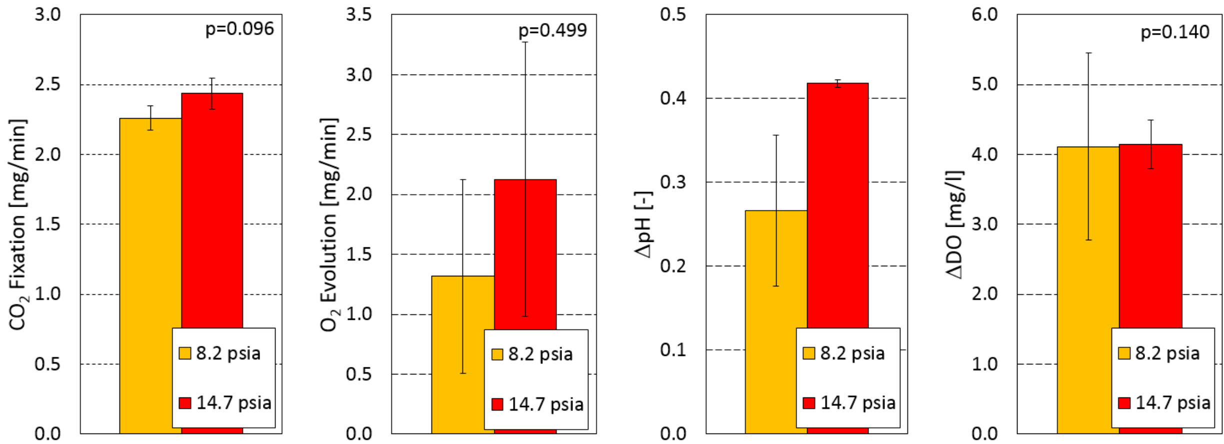

Figure 30: Average metabolic rates between the two experiment conditions measured in the gaseous phase (CO_2 fixation, O_2 evolution) and in the liquid phase (ΔpH and ΔDO). Error bars represent standard deviation with the p-value shown in the upper right.

Data can be compared to previous investigations on *Chlorella vulgaris* demonstrating an oxygen evolution rate between 2.1 and 7.7 $\frac{g}{l \cdot d}$ (Ammann and Lynch, 1965; Bovee et al., 1962; Fan et al., 2007; Javanmardian and Palsson, 1991; Lee and Palsson, 1994; Lee, 1999). The current experiment, with an oxygen evolution rate between 0.5 and 1.1 $\frac{g}{l \cdot d}$ (average 1.0 $\frac{g}{l \cdot d}$), is on the lower end of the reported oxygen evolution rates, but still in the same order of magnitude. Major items that have contributed to this low efficiency are thought to be non-optimal light distribution design as well as using a non-axenic culture, providing an increased metabolic activity to a diverse microbiome. It should also be pointed out that a limited number of studies have demonstrated much higher oxygen evolution rates, between 21.8 and 53.1 $\frac{g}{l \cdot d}$, using high-density photobioreactor designs (Hannan, 1964; Salisbury et al., 1997; Shuler and Affens, 1970).

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6.4.4 Metagenomic sequencing

It was shown that several genera of the family of Chlorellaceae are present in the algal culture. Mapping results identified *Chlorella vulgaris*, *C. variabilis*, and *Parachlorella kessleri* in all 3 samples where the latter was by far the one with the most gene abundance. Some of the samples also matched with lower genera level of *Auxenochlorella protothecoides*, *Marinichlorella sp.* KAS603, *Chlorella sorokiniana*, *Chlorella sp.* ArM0029B, and *Micractinium reisseri*. The green alga *Chlamydomonas reinhardtii* was also recognized in the analysis.

The 35 most abundant genome reads for each sample where analyzed and classified. In addition to the Chlorellaceae, reads also mapped to the phylum of Proteobacteria, Chlorophyta, and Chordata. It is noteworthy to point out that not a single read of those mapped to the phylum of cyanobacteria. This is valuable as the observation in photosynthetic metabolism can be traced back solely to Chlorellaceae. Only trace elements of one other species (*Coccomyxa subellipsoidea C-169*) out of the class of Trebouxiophyceae has been found in the culture. Within the Proteobacteria, a wide diversity of bacteria was found ranging from *Mesorhizobium* over *Variovorax* to *Caulobacter* to name just a view. Most notably, *Methylobacterium oryzae* CBMB20 and *Azospirillum lipoferum* 4B and *Gluconacetobacter diazotrophicus* have been found that are reported to promote plant growth. The most common mechanisms herefore are either by facilitating resource acquisition such as nitrogen or phosphorus fixation, or by modulating plant hormone levels (Glick, 2012). The bacteria *Azospirillum* ssp. have been specifically studied with Chlorellacea as a model

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organisms for plant-bacterium interactions that have led to increased growth rates (De-Bashan et al., 2008; De-Bashan and Bashan, 2008). Nitrogen fixation of those bacteria seemed to play only a subordinate role, as the difference in nitrogen partial pressures between the two experiment conditions did not show any effect on the growth of the algae. The species *Acanthamoeba castellanii str.* Neff of the family of Acanthamoebidae is the only other eukaryotic cell that has been found in the culture. Lastly, some reads mapped to the Chordata *Danio rerio* (zebrafish) and the Mammalia *Pantholops hodgsonii* (tibetan antelope), *Homo sapiens* (human), and *Mustela putorius furo* (ferret). Due to the method of selecting only 5000 random reads for the whole genome analysis, there is a high likelihood for probability influences in the mapping results.

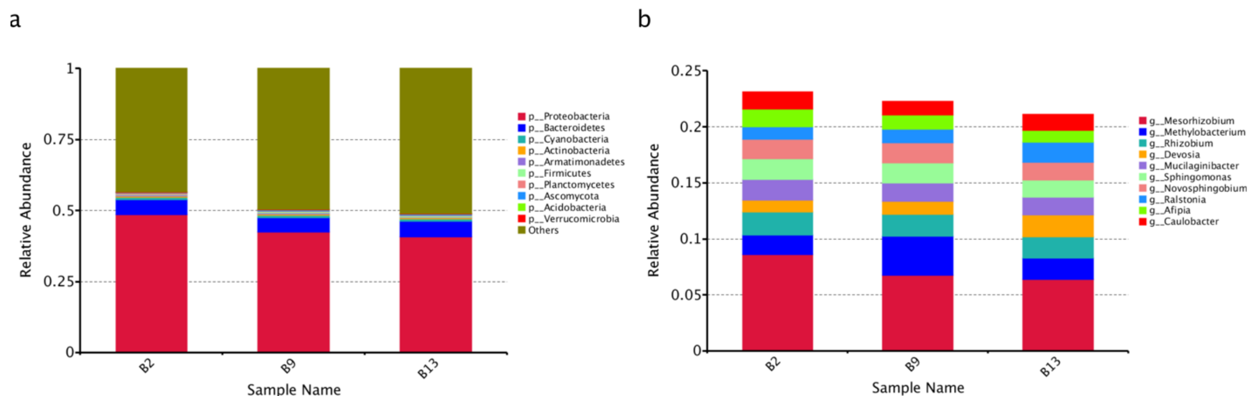

Figure 31: Species relative abundance bar plot in phylum level (a) and genus level (b)

Metagenomic analysis of the contamination after removal of the host sequence showed a high degree of correlation between the different experiment runs as shown in Figure 31. This consistency of the algal culture over time allows a comparison of the results between the different experiment runs by excluding any changes in

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biological composition. It also points to the diversity contained in non-axenic cultures, even when sterile technique is employed.

The low oxygen evolution as mentioned in 0 can be underlined by the function annotation shown in Figure 32. It is shown that the single most abundant gene function is related to metabolism in the contamination. This metabolism in the culture reduces the photosynthetic activity of the reactor as part of the evolved oxygen is directly consumed by the bacteria.

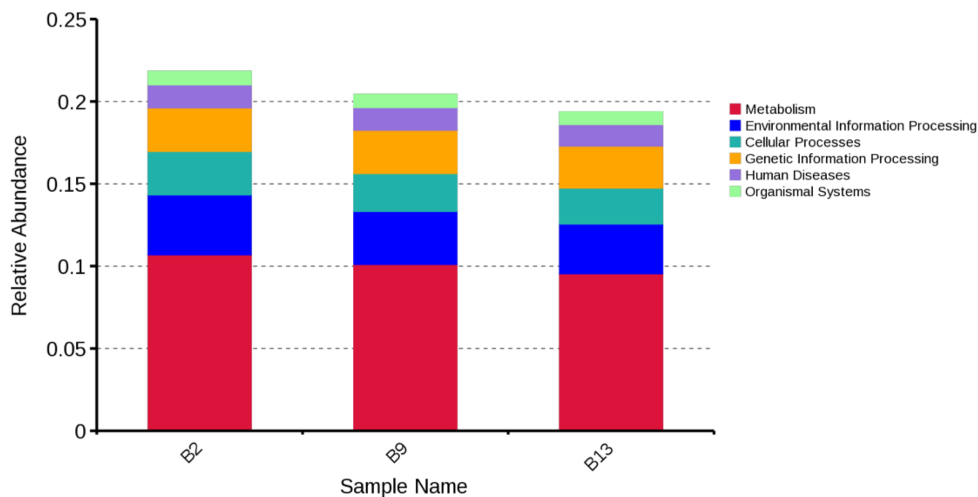

Figure 32: Function annotation relative abundance using the KEGG database

In conclusion, the metagenomic analysis identified contamination of a 3-year culture representative of conditions in an operational environment such as a spacecraft. Such a combination of metabolic measurements with a metagenomic analysis has, to the best of our knowledge, not been performed before. The analysis also increased the value of the metabolic measurements, as it showed comparability between the experiment cultures, but also verified that no other photosynthetic organisms except green algae were present in the experiments. The contamination

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leading to increased metabolic activity, as well as the existence of a variety of closely related algae from the family of Chlorellaceae, allows a new angle for interpreting different algal metabolism studies. It provides a cautionary example when interpreting results between strains of "the same" algal taxon.

6.4.5 Other observations

Minor failures were encountered during these experiments. However, it should be pointed out that most of them were of mechanical rather than biological nature. Most failures such as erroneous sensor readings, leaks, or empty consumables could be resolved quickly without impact to the experiment. A major source of component degradation over time was the use of a deliquescent and corrosive calcium chloride humidity absorber that is required for reliable gas composition measurements. Other humidity absorber chemicals such as silicon dioxide or Drierite where not suitable due to their own absorbance of either carbon dioxide, oxygen, or both (Elia et al., 1986).

Algae have proven to show very robust growth under different pressures, but also during handling. However, it should be noted that algae have shown flocculation (see Figure 33) due to sudden pressure changes (from 14.7 to 8.2 psia in less than a second) at high cell densities.

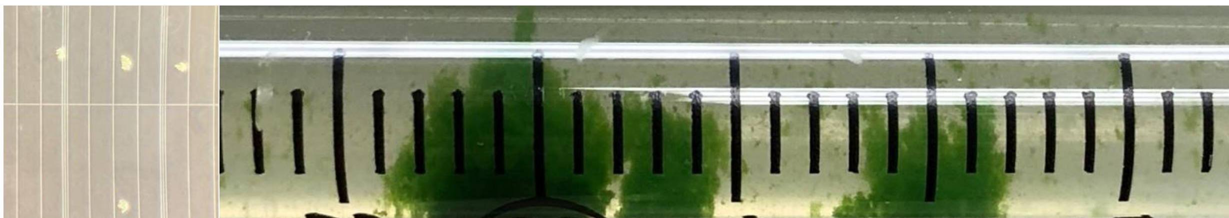

Figure 33: Flocculation as seen under the microscope and in the sample syringe

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6.5 Conclusion

In this study green algae were grown under altered atmospheric pressures while maintaining the partial pressures of carbon dioxide and oxygen to simulate NASA proposed exploration atmosphere conditions for future long-duration human spaceflight missions.

A novel experimental photobioreactor was developed that supports flow-through studies while actively controlling atmospheric pressure and composition. The applicability of the test setup, however, is not just limited to the exploration atmosphere, but can be universally used for studies involving specific partial and total pressures of a variety of gases.

While a hypothesized increased performance could not be verified, it was shown that the nitrogen partial pressure neither benefits nor limits algal growth. The cause for this observation is thought to be the continuous mixture of algal cells in culture which allowed diffusion to not be the limiting factor in algal growth. Performance of the algal culture was shown to be similar under the exploration atmosphere and standard sea level conditions both by cell growth (cell population density and optical density) as well as online metabolic measurements (cO_2, cCO_2, ΔpH, ΔDO). As long as the algal culture is continuously well mixed, the results are hypothesized to be applicable both in microgravity as well as in altered gravity (Moon, Mars), which has to be verified in future studies.

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To the best of our knowledge, this study has combined a metagenomic analysis with continuous metabolic measurements in algae for the first time. The characterization of contamination that developed in a 3-year non-axenic culture is intended to be representative for a culture in an operational human-tended setting. This is important, as the biological composition has direct influences on the metabolic activity and allows a new angle for interpreting different algal metabolism studies with a wide range of oxygen evolution rates.

6.6 Resultant publications and presentations

Journal Papers

1. **T. Niederwieser**, P. Kociolek, and D. Klaus, "Effect of altered nitrogen partial pressure on Chlorellaceae for spaceflight applications," Algal Research [IN PREPARATION]

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7 Effects of relevant spacecraft chemical contaminants on the health of algal cultures

7.1 Introduction

In addition to biological contamination, chemical contamination is a potential thread for the long-term stability of algal cultures. This is of importance as life support systems are a life critical system. Within the unique closed and operational environment of a crewed spacecraft, particular focus is given to airborne contaminants. The airborne contaminant environment is well characterized onboard the ISS and controlled to levels below those given in Spacecraft Maximum Allowable Concentrations for Airborne Particles (SMAC) and Spacecraft Water Exposure Guidelines for Selected Waterborne Contaminants (SWEG) (NASA, 2017e, 2017f). If algal photobioreactors become implemented in closed spacecraft life support systems, it will be unlikely that sterility can be maintained in the algal photobioreactor due to required human maintenance, but also due to the size of algal photobioreactors. For future spacecraft implementation it is therefore important to characterize the effects of chemical compounds that have an effect on the health of algal cultures so that appropriate requirements or safety measures can be implemented.

7.2 Background

The SMAC, for example, lists a total of 55 compounds with maximum allowed limits due to human health effects. Out of those 55 tracked compounds, 5 relevant and readily available contaminants were chosen. Additionally, 4 relevant compounds

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not listed in the NASA documents were added to the list such as calcium chloride (used as dehumidifier), lithium hydroxide (used as carbon dioxide absorbent), bleach (used as disinfectant), as well as Liquinox (used as cleaning agent).

Table 14. Selected contaminant details for investigations

Compound	Max. Exposure Limit [mg/m^3]	Manufacturer	Identifier
Bleach	N/A	Clorox	Concentrated Germicidal Bleach (30966)
Liquinox	N/A	Alconox	Liquinox
Calcium Chloride	N/A	HomeScienceTools	CH-CACL500
Lithium Hydroxide	N/A	Sigma-Aldrich	442410-500G-A
Silica Gel	N/A	Chem Source, Inc.	Beaded, Grade 20, Type II (SG20004)
Ethanol	10,000 (1hr)	Decon Labs	Ethanol 200 PROOF (2701)
Methanol	260 (1hr)	Fisher Scientific	A433F-1GAL
Formaldehyde	1 (1hr)	Sigma-Aldrich	Formalin Solution 10 % (HT5012-60ML)
Ammonia Sulfate	20 (1hr)	Fisher Scientific	A702-500
Control	-	-	-

7.3 Material and methods

Algae cultures are prepared in autoclaved 200 ml flasks by adding 10 ml of algal stock culture (as described in chapter 6.3.1) out of the logarithmic growth phase to a mixture of 150 ml of ultrapure water and 3 ml of concentrated Bolds Basic Medium (BBM) as growth media (PhytoTechnology Laboratories, BBM Solution 50x, B1650). Subsequently, 75 mg of each investigated compound were added to the stock cultures. This number was both chosen as it lies above the specific NASA exposure limits, but also represented a number that could be reliably measured, especially for the granular compounds. It should be noted that Formaldehyde was procured in diluted form with a concentration of 10% and therefore 750 mg where used instead. Details for the nine specific compounds can be found in Table 14. The flasks were covered with a breathable parafilm membrane to avoid the exchange of solid or liquid

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contaminants while still allowing gas transfer of oxygen and carbon dioxide across the film.

Incubation was done for 25 weeks in ambient lab conditions at a temperature of 25 °C and standard atmospheric conditions in Boulder (~85 kPa, 21 % oxygen, 0.04 % carbon dioxide. Cultures were illuminated continuously with a cool white LED lighting at a PAR intensity of 40 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

Macroscopic pictures of the algal culture where done horizontally in front of a light box (Logan Electric, #810 Desk-Top Light Box) using a DSLR camera (Nikon Inc., D3400). For microscopic pictures, 20 μl out of the well-mixed algal culture where diluted in 50 μl of methylene blue (Caroline Biological Supplies, Methylene Blue Solution 875795) and let sit for a minimum of 3 min. Subsequently, a 20 μl sample was taken and spread onto a hemocytometer (Hausser Scientific, Reichert Bright-Line, 1940). Investigation was performed using an inverted microscope (Nikon Instruments Inc., Eclipse TS100) with a magnification of 20x (Nikon Instruments Inc., CFI S Plan Fluor ELWD ADM 20X) and recorded with a HD CCD camera (Allied Vision Technologies, PROSILICA GX 1910).

7.4 Results

As a first observation it should be noted that cultures with calcium chloride, silica gel, ethanol, methanol, ammonium sulfate, as well as the control (no contamination) developed from slight green into vibrant green colors indicating healthy cell population growth in those cultures. The reaction of the ten samples to

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the chemical contaminants at the end of the 25-week period are shown in Figure 34. It can be seen that cultures with calcium chloride, silica gel, and ammonia sulfate show the same color as the control culture. From the microscopic pictures it can be seen that the cells are unicellular and not stained indicating viable algal cells.

Algal cells treated with Liquinox, lithium hydroxide, and formaldehyde changed the color slowly to transparent and became fully clear after about two weeks of exposure. Additionally, the algal cells clumped together and formed visible structures in the media. Bleach changed the color of the algal culture to clear within an hour of exposure. Different than the clear cultures reported before, bleach did not make the algal cells flocculate. The color change to clear indicates the loss of green chlorophyll and with that any ability to conduct photosynthesis which ultimately leads to the death of the cell. This is further supported by the observation of dark blue stained cells indicating non-viable cells in the four clear cultures.

The cultures with methanol and ethanol both turned more opaque in which ethanol also changed color into a visibly darker green. Under microscopic investigation, flocculation was visible in methanol. Interestingly, there is a clear distinction between viable and non-viable cells visible within the flocculation indicating that a fraction of the algal culture was able to sustain the methanol contamination. Even though not flocculated, ethanol also shows clear fragments of blue-stained and therefore non-viable biological material. On the other hand, there are also viable unicellular algae. Past the two-week duration, no color change in any of the algal cultures was observed.

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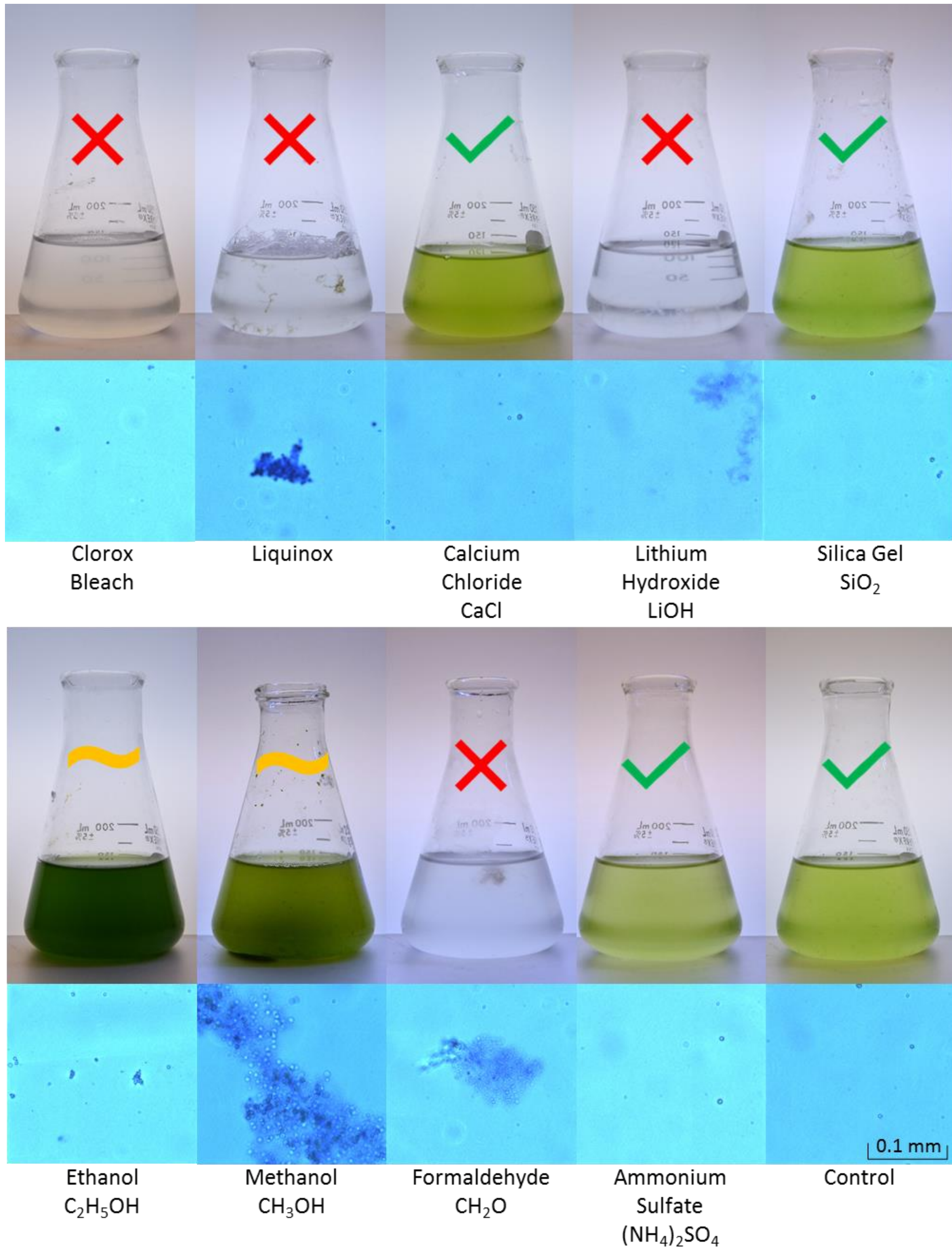

Figure 34: Macroscopic and microscopic images of the algal cultures after 25 weeks of exposure

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It can be concluded that reaction of algal cultures to selected chemical contamination is diverse and specific on the compound. Results range from immediate crash (bleach), over slow degradation (Liquinox, lithium hydroxide, formaldehyde), to alteration (ethanol, methanol) of the culture. No effect has been demonstrated of some compounds (calcium chloride, silica gel, ammonia sulfate).

7.5 Resultant outreach component

Undergraduate Involvement

1. The BOLD Center, Spring Break for Research, Emily Weidenfeller March 2018

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8 First-order feasibility study of integrating an algal photobioreactor into a spacecraft

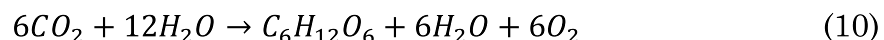
Even though the concept of bioregenerative systems has been discussed since the beginning of human spaceflight, a mass and volume-efficient implementation of a biological life support system has not yet been implemented into a spacecraft. This study reviews the different optimization criteria needed to make a photobioreactor as mass and volume-efficient as possible and assesses the feasibility to integrate an algal photobioreactor into a spacecraft from a mass and consumable perspective. The predominant average mass streams from human metabolism that have to be accommodated by a life support system are presented in Table 15.

Table 15: Human metabolism (Selection) (Anderson et al., 2015)

Interface	Balance [kg/CM-day]
Oxygen	+0.816
Carbon Dioxide	-1.040
Food	+1.510
Water Consumed	+2.500
Respired/Perspired Water	-1.900
Solid Waste	-0.110
Urine	-1.600

8.1 Background

Through photosynthesis within the chloroplast, algae fix carbon dioxide in glucose and evolve oxygen from water per equation 10.



$$6CO_2 + 12H_2O \rightarrow C_6H_{12}O_6 + 6H_2O + 6O_2 \quad (10)$$

This correlation shows that algae are capable of achieving a maximum theoretic photosynthetic quotient (defined as the molar ratio between oxygen released and

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carbon dioxide absorbed) of 1.0. In reality, there are a variety of processes occurring in an algal cell including cell respiration, nutrient uptake, and carbohydrate processing that can influence this quotient through further gas exchange. The resulting overall balance is known as the apparent photosynthetic quotient which can achieve long-term (6 months) values between 0.3 and 1.8 (Ammann and Lynch, 1965; Britain and Press, 1991; Burris, 1981). Through informed selection of species, genetic engineering, growth media optimization, and optimizing the environmental parameters, the photosynthetic quotient can theoretically be matched to the average human respiratory quotient of about 0.92 (Anderson et al., 2015).

The maximum theoretical achievable efficiency to convert incident light energy into carbohydrates under natural conditions was calculated to be 4.3 % for C3 plants, 5.3 % for C4 plants, and 5.4 % for microalgal mass cultures (Melis, 2009; Tredici, 2010; Zhu et al., 2008). However, actual experimental long-term values for higher plants have seldom exceeded 1 % (Walker, 2009), whereas experimental long-term values for algae of 4 % have been documented (Tredici, 2010). Different from growth in natural conditions, which are usually of interest to the terrestrial food and biofuel industry, algae in spacecraft applications will be grown in high-density photobioreactors that tightly control the environmental conditions. This shift in optimization goals opens new opportunities for algal growth. As natural sunlight only contains about 45 % of usable light for photosynthesis, photobioreactors that provide selected wavelengths can theoretically achieve photosynthetic efficiencies of 12 % for microalgae (Tredici, 2010).

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There have been a multitude of studies looking at the oxygen evolution of algae, both at large and small scales. In Table 16, a selection of different published values is shown. Most notably, studies performed by Boeing and BIOS-1 successfully supported one human in terms of air revitalization for several days (Bovee et al., 1962; Salisbury et al., 1997).

It is important to point out that there is quite a variation in oxygen evolution between the different studies due to different species, reactor types, reactor volumes, and cell population densities to name just a few. In most studies, it was pointed out that the algae were limited by lighting, gas transfer, or growth medium, but surprisingly never the biological potential under optimum conditions. In order to differentiate the success of the three highest performing experiments (Hannan and Patouillet, 1963; Salisbury et al., 1997; Shuler and Affens, 1970), reactor types, penetration depth and gas exchange were examined in further detail. It should be pointed out that the reactor description of the BIOS-1 experiment is only available on a very top level. Three different reactor types have been used: First, flat panel photobioreactors, secondly, internally-lit and water-jacketed photobioreactors, and thirdly cylinders with uniquely designed lighting distributions. No correlation between reactor type and oxygen evolution was found. There is a general trend that the penetration depth is decreasing with higher oxygen production rates, but Lee and Palsson (1999) as well as Ammann and Lynch (1965) have not yielded a high oxygen evolution rates despite low penetration depth which caused us to also consider the gas transfer technology. All of those 4 studies used internal sparging to transfer gas

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between supply and culture. However, different from the studies with higher productivity, Ammann and Lynch (1965) as well as Lee and Palsson (1994) do not mention the use of a diffuser while using comparable concentrations and flow rates. This could indicate a limitation of the algal cultures in the ability to resupply carbon dioxide and remove oxygen.

Table 16: Review of experimentally derived oxygen evolution rates found in the literature

Reference	Year	Volume [liter]	O_2 evolution [g/liter-day]
Boeing (Bovee et al., 1962)	1962	380.0	2.1
Ammann (Ammann and Lynch, 1965)	1965	0.7	3.7
Fan (Fan et al., 2007)	2007	5.6	4.5
Palsson (Javanmardian and Palsson, 1992)	1991	0.6	4.6
Lee (Lee and Palsson, 1994)	1994	0.1	7.7
Lee (Lee, 1999)	1999	0.1	7.7
Hannan (Hannan and Patouillet, 1963)	1964	6.2	21.8
BIOS-1 (Salisbury et al., 1997)	1997	18.0	45.3
Shuler (Shuler and Affens, 1970)	1970	0.8	53.1

8.2 Methods

This feasibility study establishes the biological and engineering needs that allow fast growth and productivity at high cell densities of algae in general. The term algae is hereby widely used for both cyanobacteria and green algae. Not one specific species is chosen in this study, as probably more than one species will be implemented and not all parameters could be found for each species. As example, common species whose values for this study are derived from literature and tests are *Chlorella vulgaris*, *Chlorella sorikiniana*, *Scenedesmus acuminatus,* but also cyanobacteria such as *Spirulina platensis*. The requirements are then used to develop a conceptual implementation of an algal photobioreactor into a spacecraft. From this concept, key

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characteristics such as mass, power, volume, heat load, and the required logistics supplies are determined. Finally, the established algal design concept is compared to current non-biological state-of-the-art systems onboard the International Space Station (ISS).

8.3 Feasibility analysis

It is a common belief that algal life support systems are voluminous systems that exceed the mass and volume constraints of a spacecraft. From the values in Table 16 it can be seen that the daily oxygen requirement for one crewmember of 0.816 kg (see Table 15) can be achieved, using the highest demonstrated algal oxygen evolution rate of 53.1 g per liter culture per day. This results to a total algal culture volume of only 15 liters to completely support one human in terms of air revitalization.

Requirement 1: Provide a culture volume of 15 liters.

In order to support such a high-density and high-productivity photobioreactor onboard a spacecraft, it is crucial to avoid growth-limiting conditions by supporting the biology with an optimized engineering design. The most critical parameters of any photobioreactor are lighting, gas transfer, growth media, and harvesting, which are discussed in that order below.

8.3.1 Lighting

Since light is the energy source for photosynthesis, an optimized light distribution is crucial to design a photobioreactor. Light can be described through the three characteristics of spectrum, intensity, and cycle. Janssen has shown that a

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variety of species grow well under continuous lighting which is crucial to design a continuously operating life support system (Janssen, 2002).

Both the typical absorption spectrum (the band of wavelengths algal cells take up as light energy) and the photosynthetic action spectrum (the band of wavelengths cells use for photosynthetic activity) peak around 670 nm (red light) (Nakajima et al., 2015). Modern LED lights, optimized for horticulture use, are capable of providing light in a narrow bandwidth around 670 nm, reducing the losses due to unused wavelengths (e.g. 55 % for sunlight).

Requirement 2: Provide light at a wavelength of 670 nm.

Depending on the light intensity, photosynthesis activity differs in 3 different areas: First, at low light intensities photosynthetic activity increases linearly with increasing light intensities. Secondly, light impact plateaus once saturation is reached around 200-400 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (equal to about 36-71 W/m^2) for most species (Bhola et al., 2011; Degen et al., 2001; Melis, 2009). Lastly, algae reach photoinhibition at light intensities exceeding 1400 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (equal to about 250 W/m^2) where biological activity suddenly drops off.

Requirement 3: Provide light at an intensity between 36 and 250 W/m^2.

Light intensity decreases with the inverse of the distance squared from the source. Lee et al. showed a relation between light intensity at the surface, cell population density, and light penetration depth (Lee, 1999). At a surface intensity of

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250 W/m^2 and a cell concentration of 2*10^8 cells/ml, the penetration depth is 2.5 mm. This is a conservative estimation as in reality algal cells can be forced into the turbulent flow regime which exposes more algal cells to light due to cells constantly traveling between lit and shadowed regions.

Requirement 4: Provide a maximum penetration depth of 2.5 mm.

8.3.2 Membrane Characteristics

In a high-efficiency photobioreactor it is a challenge to maintain a sufficient transfer rate of carbon dioxide into the reactor and oxygen out of the reactor in order to not limit algal growth. Sparging (the injection of gas at the bottom of the reactor with gravity-driven buoyancy of air bubbles through the liquid) is the most-commonly used method for terrestrial applications. However, in a microgravity environment where gas bubbles will not rise, it is not feasible to use this method and most likely a membrane approach will be needed for gas transfer. The material selected must have a high permeability to both oxygen and carbon dioxide, but contain the liquid algal culture. Table 17 shows a summary of potential membrane materials and their characteristics.

Table 17: Membrane permeability for oxygen and carbon dioxide for a variety of materials (Cole-Parmer, 2015)

Material	Permeability $\left[x10^{-10}\,\frac{cm^3\,mm}{s\,cm^2\,cmHg}\right]$	
	CO_2	O_2
Silicone	20,132	7,961
Tygon	4,840	980
Natural Rubber	1,311	307
Polyethylene	280	60

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Based on these characteristics for the reactor, silicone is used for the following scaling as a membrane material. The required daily transfer for one crewmember across the membrane of 0.816 kg oxygen and 1.040 kg carbon dioxide according to Table 15 calculates to a volume of 0.416 m^3 and 0.728 m^3 at STP using the ideal gas equation. Calculating the required membrane area requires also knowledge of the partial pressure gradient across the membrane. The most straightforward system is directing the flow of cabin air over one side of the membrane and having the algal culture on the other side. Assuming a standard cabin environment of 14.7 psia/101 kPa with 21 % (3.1 psia/21 kPa) oxygen and 0.26 % (0.04 psia/0.3 kPa) carbon dioxide (Hurlbert et al., 2012) on one side and the algal culture at the same pressure of 14.7 psia/101 kPa with 0.03 psia/0.2 kPa carbon dioxide and 3.2 psia/22 kPa oxygen on the other side of a 0.127 mm thick silicone membrane results in a membrane surface area of 709 m^2. This result was calculated using Equation 11, where A is the membrane area, V the gas transfer volume (at STP), d the thickness of the membrane, J the permeability of the material, Δp the pressure differential across the membrane, and t the time of operation.

$$A = \frac{V\,d}{J\,\Delta p\,t} \tag{11}$$

The result is problematic not only because carbon dioxide, in order to not be growth limiting, needs to be at a concentration equal to a partial pressure of 0.735 psia/5 kPa (Bhola et al., 2011; Chinnasamy et al., 2009; Singh and Singh, 2014), but also because current small-scale hollow-fiber membranes have a surface area of only a few square meters (Permselect, 2018). Even the human lung as a three-

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dimensional membrane has an approximate surface area of only 75 m^2. Increasing the pressure of the cabin atmosphere locally at the membrane in a pressurized enclosure leads to an improvement in carbon dioxide transfer while at the same time leading to a decrease in oxygen transfer making this option not feasible.

Incorporating a carbon dioxide concentrator provides a way to decrease the surface area while maintaining the required gas transfer rates. Using equation 11 above, an increase of partial pressure to 14.0 psia/96 kPa carbon dioxide at a 95 % concentrated gas stream on the one side and the algal culture at the same pressure of 14.7 psia/101 kPa with 0.735 psia/5 kPa carbon dioxide and 3.1 psia/ 21 kPa oxygen on the other side of a 0.127 mm thick silicone membrane results in a surface area of only 4.9 m^2. This drops the surface area significantly into the realm of feasibility but mandates a carbon dioxide concentrator.

Requirement 5: Provide a carbon dioxide input stream of >95 %.

Requirement 6: Provide a surface area of 4.9 m^2 silicone for gas exchange.

8.3.3 Growth media

Human urine collected in a spacecraft waste stream can serve as a microalgal growth medium due to its high nitrogen and phosphorus content. Several studies have been performed with both diluted and non-diluted urine as growth media and have achieved good growth (Jaatinen et al., 2016; Tuantet et al., 2014b, 2014a). Even though urine has a nitrogen to phosphorus atomic ratio between N/P=30 and 40,

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which is higher than the known Redfield ratio of N/P=16 for optimum growth in microalgae (Yoshida et al., 1958), growth rates of 0.104 per hour have been demonstrated on non-diluted female urine (Tuantet et al., 2014a). This value can almost be doubled by diluting the urine in water between 2x and 3x, as well as adding minor trace elements, most effectively Mg^{2+} at a concentration of 1.5 mg Mg^{2+} per gram of biomass.

Tuantet et al. have demonstrated removal rates of 0.15 g phosphorus per liter per day and 1.3 g nitrogen per liters per day for a total duration of eight months using the green algae *Chlorella sorokiniana* in 3x diluted urine. However, these rates were only achieved through supplementation of magnesium and phosphorus. Urine has a total nitrogen content of about 6,340 mg/l, a phosphorus content of about 401 mg/l (Tuantet et al., 2014a), and a human releases on average about 1.6 liters of urine per day. In order to not limit the 15-litres algal culture in terms of nutrient supply, a urine input of 1.95 persons would be needed for nitrogen and 4.28 persons for phosphorus, while only sustaining one person in terms of carbon dioxide and oxygen.

Requirement 7: Provide the capability to inject 1.6 kg of urine per day.

8.3.4 Harvesting

Carbon dioxide is continuously produced by the crew and therefore has to be continuously processed to maintain allowable atmospheric conditions, along with oxygen exchange, which can be accomplished by an algal culture as described above.

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Urine as the growth media can be simply injected into the culture, but some control system is needed to keep the culture near a constant cell population density.

As described in section 8.3.1, a cell population density of about $2*10^8$ cells/ml is a good compromise between high-density cultures and light penetration depth. Using an average cell dry weight of $2.24*10^{-11}$ g/cell (Hu, 2014), this correlates to a cell dry weight density of 4.5 g/l. However, other studies have experimentally measured that value to be around 15 g/l (Chioccioli et al., 2014; Hu et al., 1998; Tuantet et al., 2014a). The correlating dry biomass productivity is also around 15 g/l-day totaling to 225 g/d with a 15-liter photobioreactor.

Requirement 8: Provide a biomass harvesting capability of 225 g/d.

8.4 Results

Based on the requirements derived in section 8.3, an implementation concept and design are established. Due to the excessive membrane area requirement at ambient carbon dioxide levels, a direct exchange loop between the crew and photobioreactor does not satisfy the needs of a life support system. Instead, cabin air with ambient carbon dioxide levels is first fed to a carbon dioxide removal system, as proposed in Figure 35, to concentrate carbon dioxide.

This absorbs the carbon dioxide either through current systems onboard the ISS such as zeolite or solid amine beds or other innovative means. Cabin air with low carbon dioxide content is fed back to the cabin, while concentrated carbon dioxide is fed to the algal photobioreactor. Through photosynthesis, carbon dioxide is absorbed

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and converted into higher order carbohydrates while oxygen evolves from water. As the carbon dioxide stream likely cannot be absorbed completely, a stream of high content oxygen with traces of carbon dioxide emerges from the reactor that, due to the residual carbon dioxide, should not be returned to the cabin. Hence, the air stream from the photobioreactor will be fed back and mixed with the feed stream to the carbon dioxide removal system. This recirculation loop allows the separation of oxygen and carbon dioxide so that oxygen can be provided to the cabin and carbon dioxide recirculated into the photobioreactor.

Additionally, the photobioreactor will need makeup water and urine feedstock from the crew. Urine can be inserted without pre-processing and humidity can be removed from the air through systems such as cold plates. Lastly, the biomass must pass through a harvesting device to maintain a constant cell density within the photobioreactor. After further processing, biomass as well as water can ultimately be provided to the crewmember for nutrition and hydration. In addition to these mentioned mass streams, the photobioreactor requires electrical power and emits heat energy that must be removed.

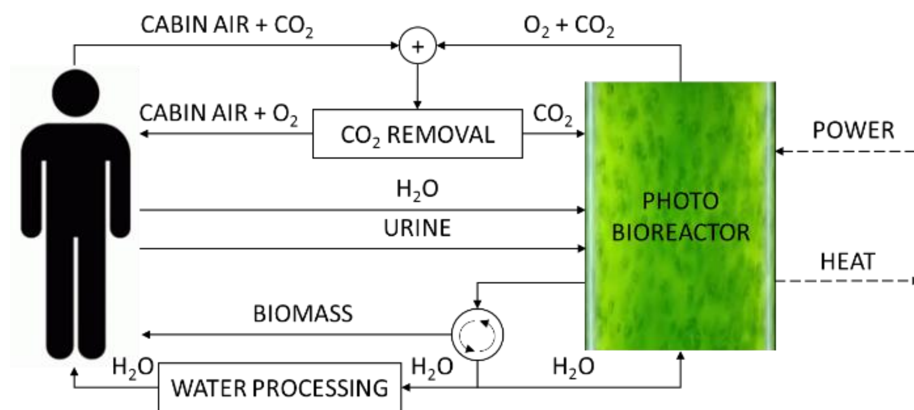

Figure 35: Conceptual approach of implementing an algal photobioreactor into a spacecraft

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8.4.1 Conceptual packaging

In order to satisfy mass, power, and volume budget limits, packing densities must next be derived for the design. A flat panel photobioreactor sheet concept is illustrated in Figure 36.

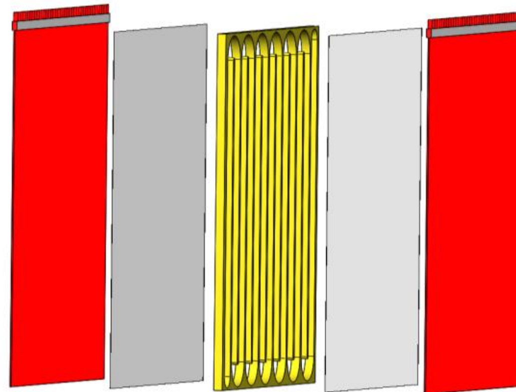

Figure 36: Flat panel photobioreactor sheet. (yellow: Polysulfone reactor sheet, grey: silicone membrane, red: fibre optics panel)

The 20 x 20 cm center panel is made from biocompatible polysulfone as structure that also determines the algal flow path for good mixing. Through 0.127 mm thick silicone membranes (e.g. Speciality Silicone Membranes, M832), carbon dioxide and oxygen diffuse in and out of the algal culture. As the polysulfone is 5 mm thick, light has to be provided from both sides to achieve full light penetration. The initial idea was to sandwich the polysulfone sheets with LED populated circuit boards. However, the opening angle of the LED would have required large gaps between the illumination panel and the actual algal culture making the assembly volume unnecessarily large. Additionally, heat removal would have become a challenge. Instead, light-distributing fiber optic panels (Lumitex, Uniglo) were chosen to both transport light from externally heat sunk LEDs to the reactor and to create a uniform

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light field. With a height of only 0.8 mm these panels are optimal for sandwiching between the membrane clad polysulfone sheets. A short gap is maintained between the fiber optics panel and the membrane to allow gas to flow across the membrane as shown in Figure 37. Fans are included to assure proper gas mixing between the sheets and prevent the build-up of any carbon dioxide or oxygen pockets.

LEDs that emit light in a narrow spectrum around the wavelength of 670 nm (e.g. CREE, XP-E HE Photo Red) feed the fiber optic panels from the top. A total of 2144 LEDs are mounted on and powered from a circuit board that is heat sunk to a water-cooled aluminum plate for heat removal as seen in Figure 37.

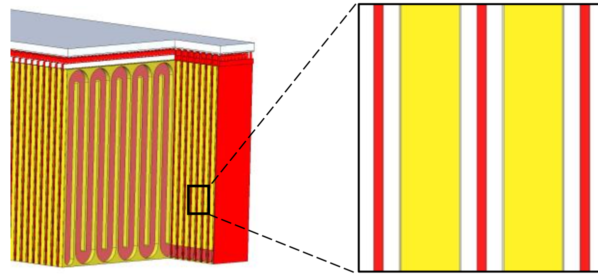

Figure 37: Cut through of the algal photobioreactor pack consisting out of stacked membrane-clad polysulfone sheets and fibre optic panels (shown in the zoomed in section). The LED illumination panel can be seen spreading across all sheets on top

Each polysulfone sheet can hold a culture volume of 163 ml. In order to reach a total culture of 15 litres, 92 sheets have to be stacked to a pack with the dimensions of 40 x 35 x 23 cm. For illustration purposes such a pack is shown within a standard middeck locker in Figure 38. The total algal surface for both lighting and gas exchange is 6 m^2. All flat panels are connected with each other via tubing to create a loop that algae are pumped through using a peristaltic pump (e.g. Watson Marlow,

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PMD24C). In order to maintain a constant cell density, the algae have to complete the 15-litres cycle at least once per doubling time of about twelve hours. Hence, the minimum capacity of the pump has to be 1.25 liters per hour but likely will be higher in operation to prevent surface adherence and assure proper mixing.

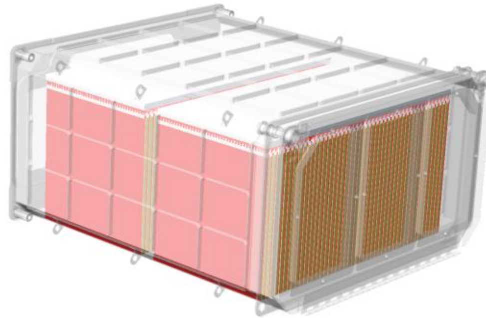

Figure 38: Algal cell pack sized for air revitalization for one crewmember shown in a standard middeck locker for comparison

Different harvesting methods are available, such as centrifugation, filtration, or hydro cyclonic harvesting, but they all have their challenges in terms of complexity, consumables, or filtering efficiency. One promising technology that currently resides at low TRL is ultrasonic harvesting. Applying an ultrasonic wave to a tube that algae are being pumped through creates centres of high and low algal densities. Innovative flow channels can then separate a concentrated algae stream.

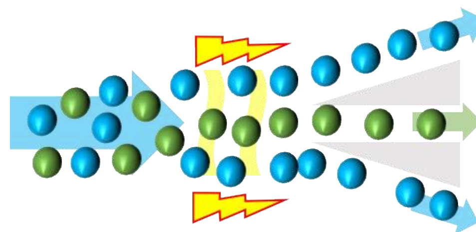

Figure 39: Simplified concept drawing of ultrasonic harvesting. A steady ultrasonic field is applied to the incoming algal suspension causing the algal cell to accumulate in the wave centres. The high concentrated algal stream can then be divided by flow division.

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As the concept of ultrasonic harvesting is low TRL, a mass and volume budget are difficult to create. However, pursuing this design is still promising as there are no moving parts and the operation can be performed continuously. Gomez has developed a laboratory scale harvesting device that was able to continuously achieve a filtration efficiency of 70 % and a concentration factor of 11.6 (Gomez, 2014). The flow rate was 25 mL/min under an energy consumption of 3.6 kWh/m^3.

8.4.2 Mass flow balance of conceptual design

Table 18: Overall mass flow balance of the photobioreactor

Material	Productivity [kg/d]
Oxygen	+0.816
Carbon Dioxide	-1.040
Biomass	+0.255
Nutrients	-0.011
Pre-processed Water	+1.580
Urine	-1.600

Since the reactor is sized to support one human in terms of air revitalization, the output and input in terms of oxygen and carbon dioxide are consequently 0.816 kg/day and 1.040 kg/day. The corresponding biomass that is produced amounts to 255 g/d which can be used as food supplement. The input of urine is limited by the human production which amounts to 1.6 kg/day on average, but the algae could actually handle 3.12 kg/day. To make up for this limitation, nutrients will have to be provided in addition to urine to get a high-density algal culture. This amounts to 9.5 g/d for nitrogen, 1.6 g/d for phosphorus, and 0.3 g/d for magnesium. The total nutrient consumption is therefore 11.4 g/d. From the 1.6 kg/d of urine, 10.1 g/d phosphorus

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nitrogen, 0.6 g/d, as well as minor amounts of other trace contaminants are taken up resulting in an output of pre-processed water of 1.589 kg/d. Even though this reactor is sized for one crewmember in terms of air revitalization it can hence be seen that the developed reactor would also support the urine processing for one crewmember. In Table 18, the total productivity of the reactor is summarized.

8.4.3 Mass

The mass was determined using a bottom-up approach with values derived from a CAD model as well as product datasheets. The driver for an algal photobioreactor is the algal culture itself at 15 kg of liquid, in addition to the actual reactor (flat panel, lighting, cold plate) with a mass of 19.3 kg. Other components such as the peristaltic pump, the air distributing fans as well as the harvesting devices have minor masses of 1.2, 1.9, and 1.0 kg respectively. Since it would be out of scope of this study to design a complete flight unit, a heuristic approach was used to determine the structural weight. From section 8.4.4, it appears that an algal photobioreactor could fit in a volume of ≤2 middeck locker equivalents. For a conservative approach, a quarter of an empty EXPRESS rack (total empty weight 356 kg) is used to estimate the structural mass and interfaces to 89 kg. The total mass estimate resulted in 127.4 kg (see Table 19).

8.4.4 Volume

Using the same approach as explained in section 8.4.3, the volume of the algal reactor was calculated to be 0.056 m^3. Since the 15-litre algal culture is contained within the reactor it does not count towards the volume budget. Pumps, harvesting

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device, and fans only make up 0.005 m^3. To account for integration losses and support structure a quarter volume of an express rack without lockers was added (0.346 m^3). The total volume estimate resulted in 0.407 m^3.

8.4.5 Power

In terms of power the LED lighting is by far the biggest consumer. 2144 LEDs require a power of 3750 W. Other continuously operating components (fan, harvesting device, peristaltic pump) only require a combined power of 172 W. The total power estimate results in 3922 W. A summary of the mass, power, and volume characteristics of the proposed algal photobioreactor are provided in Table 19 below.

Table 19: Characteristic estimates for the sub-components

Material	Mass [kg]	Volume [m^3]	Power [W]
Reactor pack	19.3	0.056	3750
Algal culture	15.0	0.015*	-
Peristaltic Pump	1.2	0.002	48
Fan	1.9	0.001	70
Harvesting Device	1.0	0.002	54
Support Structure	89.0	0.346	0
Total	127.4	0.407	3922

*included already in the reactor pack volume

It also should be pointed out that the 2144 LEDs have an efficiency of about 40 % to convert the electrical energy into light energy. The remaining energy is converted into heat energy that has to be accommodated by the spacecraft. Heat rejection by other components such as fan, peristaltic pumps, or the harvesting device are negligible in comparison to the LEDs.

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8.5 Discussion

The majority of results in this study have been derived from experimental data collected using the green algae *Chlorella vulgaris*, as it is a very robust and well documented species. However, other species that have high growth rates and have been tested on urine as growth media are for example *Chlorella sorikiniana*, *Scenedesmus acuminatus,* but also cyanobacteria such as *Spirulina platensis* (Jaatinen et al., 2016). For a high system level robustness, it might even be beneficial to implement a wide variety of species out of different kingdoms that adapt and perform differently under changes in environmental conditions.

A recent study looking at the optimum growth parameters has determined gravity, radiation, and pressure as unknown factors for photobioreactor development in spaceflight applications (T. Niederwieser et al., 2018). Varying atmospheric pressure through change of nitrogen partial pressure was shown to not have an effect on algae (Niederwieser, 2018; Orcutt et al., 1970). Algae have been flown intensively in space, however, conclusive results are still missing (Tobias Niederwieser et al., 2018b). Currently, studies looking at the effect of microgravity on both stirred and unstirred algae are underway (Keppler et al., 2018; Settles, 2018), leaving radiation effects on algae as the last uncharacterized factor from a biological perspective.

It should also be pointed out that algal cultures have the big advantage of being restarted with inoculum cultures. If, despite self-adaptations, species variation, and redundant systems, an algal culture should die off, a preserved inoculum culture of just a few milliliters can be used to start the growth of a new culture within several

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days. Obviously, emergency systems such as consumable LiOH cartridges or a redundant photobioreactor would have to be used during that time. Preservation methods for algae range from freeze-dried passive storage to frozen active storage.

8.5.1 Comparison to state-of-the art

In order to assess first-order feasibility of implementation of this algal photobioreactor concept into a spacecraft, it has to be compared to state-of-the-art systems onboard the ISS. Currently, carbon dioxide is removed from the cabin atmosphere by the Carbon Dioxide Removal Assembly (CDRA) and fed concentrated at > 95 % carbon dioxide to the Sabatier system for reduction of carbon dioxide to water and methane using hydrogen. Together with the Sabatier reactor, the electrolysis cell stack forms the Oxygen Generation Assembly (OGA).

Table 20: Specifications of the OGA (Sabatier + Electrolysis) onboard the ISS

Mass	675 kg (Jones, 2016)
Power	1.9 kW (Takada et al., 2015)
Volume	1 ISPR Rack (1.84 m^3)*
Productivity	+ 2.41 kg O_2 day (Gentry and Balistreri, 2018)
	+ 0.6 kg CH_4 day [calculated]
	(±0.30 kg H_2 day) (Gentry and Balistreri, 2018)
	- 1.65 kg CO_2 day [calculated]
	- 1.35 kg H_2O day [calculated]

*The current ISS rack volume was taken from (NASA, 1999).

The OGA electrolyzes water to oxygen for the cabin and hydrogen that is fed back to the Sabatier reactor. Only half of the carbon dioxide can be fed into the Sabatier reactor due to the stoichiometric lack of available hydrogen. The remaining carbon dioxide is vented overboard together with the methane. Urine is treated in the Urine Processor Assembly (UPA) before it is fed to the Water Processor Assembly

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(WPA) together with other wastewater to produce potable water. UPA and WPA together build the Water Processor System (WPS). Food is not grown onboard the ISS in any substantive manner. It is resupplied in preserved form and passively stored. Systems that could be directly replaced by an algal photobioreactor are the UPA as well as the OGA, consisting of Sabatier reactor and electrolysis stack. Additional mass impacts would also occur to the food subsystem, if the algae were used as a food supplement. It is important to point out that in this initial concept, however, CDRA will still be needed to provide a concentrated carbon dioxide stream and the WPA will still be needed for conversion of the pre-processed water stream to potable water.

Table 21: Specifications of the UPA onboard the ISS

Mass	500 kg (Carter et al., 2013)
Power	424 W (Holder and Hutchens, 2003)
Volume	1/2 ISPR Rack (0.92 m^3)*
Productivity	+4.18 kg H_2O day (Gentry and Balistreri, 2018)
	-4.8 kg Urine day (Anderson et al., 2015)

*The current ISS rack volume was taken from (NASA, 1999).

Table 22: Specifications of the Food subsystem normalized to 3 CM onboard the ISS (Anderson et al., 2015)

Mass	4.53 kg day (+0.75 kg packaging)
Power	N/A
Volume	0.012 m^3/day (2.8 *10^{-3} m^3/kg)

Table 23: Comparison between an algal photobioreactor and state-of-the-art ISS systems normalized to 3 CM

Algal Reactor [3 CM]		ISS Systems [OGA+UPA]
382	Mass[kg]	1,175
1.2	Volume [m^3]	2.8
11,766	Power [W]	2,324
≥90%	Oxygen recovery	42%
≥74%	Water recovery	74%
17%	Food production	0%
0.011	Logistics [kg-d]	0

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The characteristics for an algal photobioreactor (values normalized to a crew of three from Table 19) are shown on the left in Table 23 and are compared to current state-of-the-art ISS systems (summed up values from Table 20 - Table 22) listed on the right in Table 23.

It can be seen that mass and volume of the algal photobioreactor are well below current systems, but it must be pointed out that a bottom-up estimation of an algal photobioreactor is compared to a flight unit design for the current ISS systems and is therefore probably overoptimistic. However, mass and volume seem to be comparable, if not surpassing, current ISS systems. Power is identified to be the real challenge of algal photobioreactors as it is 5x higher than the comparison system, even with high efficiency LED lighting being used. Alternatives, such as using fiber optics to bring in natural light, do exist but add complexity and will be challenging to achieve the desired light intensity. Areas where photobioreactors are superior to current systems are the increased performance and potentially long-term robustness in terms of oxygen recovery as well as food production. In addition, the same system can also be used to achieve equal or higher water recovery rates than the current UPA. It is important to point out that an algal photobioreactor is one system to process three functions (carbon dioxide and urine to oxygen, biomass, and water), whereas in state-of-the-art systems one system (Sabatier and electrolysis) is taking care of the function to reduce carbon dioxide to oxygen and another system (UPA and WPA) is taking care of the function to clean wastewater.

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8.6 Conclusions

This work presents a successful first-order feasibility study of an algal photobioreactor for complete atmosphere revitalization for one crewmember. It was shown that an algal photobioreactor of 15-liter culture volume can accommodate the air revitalization and urine processing needs of one crew member completely while also providing food supplementation. This value is lower than what is typically expected in the research community. It should be pointed out that our own experimental characterizations as described in chapter 6 were not able to achieve the oxygen evolution rates to achieve this goal either as the reactor was designed for sensitivity analysis instead of an optimized photobioreactor design. Instead this feasibility study is based on other published data that have successfully demonstrated those rates in ground studies and reported in the literature. This study investigated the needed optimizations, scale factors, and implementations to transfer the technology from small scale laboratory results to a spacecraft life support system in microgravity. Potential advantages over current ISS systems (comprised of OGA and UPA) for a crew of three in terms of mass and volume are shown. A potential disadvantage is the 5x increased power requirement, mainly for the LED lighting. However, increased efficiency of oxygen recovery, water pre-processing, as well as positive effects on the food subsystem of an ECLSS, make further pursuit of this concept worthwhile to explore. The current flight-proven ISS systems CDRA and WPA are identified as essential supporting equipment that are continued to be needed with an algal life support system. Implementing an algal life support system

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into a spacecraft requires an optimized photobioreactor design in order to fully benefit from the biological potential of algae. This study derived design requirements from the biological properties of algae and proposed a novel packaging for a photobioreactor that accommodates those requirements, such as surface area, light intensity, and gas transfer.

8.6.1 Future work

The first-order feasibility analysis has shown that an algal photobioreactor is a viable concept that can theoretically be designed to achieve a high packing density that can compete with current state-of-the art ISS systems, specifically the OGA and UPA. Noteworthy findings are the doubling of the oxygen recovery efficiency on the positive side, but the fivefold increase in power consumption on the negative side. Extending this first-order feasibility analysis to dynamic simulation with additional experimental demonstration would increase the fidelity of these results in the future. The scale of one crewmember seems reasonable for laboratory implementation. Once the engineering challenges are overcome and the operational feasibility has been experimentally demonstrated, robustness and efficiency can be further optimized biologically using genetic engineering selection and/or specific species compositions. The data obtained in this work can be used to support an Equivalent System Mass (ESM) analysis or trade study for a specific spacecraft implementation. Future work should include dynamic modelling and experimental evaluation of this concept, both under terrestrial conditions and in microgravity, to increase the TRL of algal life

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support systems aimed at enabling sustainable human exploration on long-duration spaceflight missions.

8.7 Resultant publications and presentations

Journal Papers

1. **T. Niederwieser**, T. Ruck, D. Klaus, and A. Hoehn, "Feasibility study of an algal-based life support system," Acta Astronautica [IN PREPARATION]

Conference Papers (non-refereed)

1. **T. Niederwieser**, and D. Klaus, "Feasibility study of an algal-based life support system," in Int. Astronaut. Congr. Bremen, Germany, 2018 (IAC-18.A1.7.9x43517)

Posters

1. R. Wall, **T. Niederwieser**, D. Klaus, and J. Nabity, "Evaluation of agar-grown algae for environmental control and life support systems in spacecraft applications;" in 47th Int. Conf. Environ. Syst. Charleston, SC, 2017.
2. **T. Niederwieser**, "Optimization of algal photobioreactor concepts for implementation into spacecraft life support systems;" in 48th Int. Conf. Environ. Syst. Albuquerque, NM, 2018.

Presentations

1. 69th International Astronautical Congress (IAC), "Feasibility study of an algal-based life support system" Bremen, Germany, 2018.

8.8 Resultant outreach component

Graduate Involvement

1. Technical University Munich, Master's Thesis, Thomas Ruck, July 2018 to November 2018

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9 Overall conclusions

This work represents an interdisciplinary work between biology and engineering. From the biology side, algal behavior under reduced nitrogen partial pressures has been characterized as well as the effect of selected chemical contamination on the health of algal cultures. From the engineering side, a novel test bed has been developed that combines the flow-through operations with continuous measurements and altered pressures which has not been published before. At the same time, key design requirements to support optimum growth conditions have been developed and a concept to accommodate those within the mass, power, and volume budget of a spacecraft have been proposed.

9.1 Key findings

This thesis systematically identified factors affecting the implementation of an algal photobioreactor into a spacecraft life support system with the following major outcomes:

1. **Established a comprehensive set of optimum parameters governing *Chlorella vulgaris* growth in a spacecraft and rationalized further research needed on atmospheric pressure, contamination, gravity, and radiation as unique cabin environmental factors that have not yet been fully characterized in terms of influencing the growth of algae in spacecraft cabin environments.**

Potential spaceflight applications and favorable atmospheric conditions for *Chlorella vulgaris* were identified and compared to current and proposed spacecraft cabin atmospheres. Optimum growth conditions were found to be generally in line

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with most spaceflight cabin environments, especially in terms of temperature environment, reduced oxygen concentration (under the exploration atmosphere), waste water as growth medium, and pH, where only minor modifications need to be done to current spacecraft designs to accommodate optimized performance photobioreactors using *Chlorella vulgaris*. Especially the order of magnitude elevated carbon dioxide concentration compared to levels found on Earth actually poses uniquely beneficial conditions for increased algal growth efficiency. Further increases in growth rates due to increased carbon dioxide concentrations and reduced oxygen levels beyond human physiological ranges can increase algal growth, but then require an isolated photobioreactor that cannot directly interact with the spacecraft cabin. Further research is needed to characterize the effects of altered gravity, radiation, and atmospheric pressure. Current data on those parameters do not indicate reduced performance and in fact, in terms of pressure, actually indicate increased growth rates at lower pressures. Overall, the results from this review show that algae are a promising candidate for use in a BLSS for future space exploration missions, especially under the expected environmental conditions.

2. **Evolved the first comprehensive publication featuring an international and fully historical review of algal flight experiments conducted in space with their respective outcomes.**

A total of 51 algal experiments flown in space have been identified starting from the early days of space flight in 1960 through planned experiments in 2018. From this literature, it can be concluded that algae have been widely used as model

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research organisms flown in space. So far, it has been shown that the basic biological processes associated with algal growth, photosynthesis, and respiration can function in space for durations of up to 12 months. However, dynamic culture behavior in the spacecraft environment needs further assessment and quantification and, in particular, the effects of radiation have not yet been sufficiently examined. Modern analytical tools such as RNA sequencing together with in-flight health monitoring and fixation of samples should help to better characterize the overall biological response of the cells. It can be concluded that despite the large number of algal experiments conducted in space to date, our understanding of the effects of microgravity and radiation on algae remains limited.

3. **Developed and validated a novel flow-through test stand to measure algal metabolism and growth under altered gas compositions and pressures.**

This work served to design, develop, and evaluate an experimental photobioreactor, through multiple iterations, that is capable of operating within the typical environmental conditions anticipated for future space habitats. The specific interest lies in the alteration of pressure and gas composition to characterize algal performance under NASA's proposed exploration atmosphere. It was experimentally demonstrated that the algal photobioreactor can achieve growth rates and cell densities comparable to typical benchtop bubble column photobioreactors. A baseline experiment demonstrated that no confounding artifacts are introduced from the test setup itself and its variation over time, such as the day/night cycle. Challenges, including altered gas solubilities at different temperatures, low leak rates, and

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accessibility, were shown to be successfully handled. Measurements made in the gaseous stream exiting the photobioreactor record the system performance continuously throughout the experiments. Point measurements, using the sample port, can be used to measure cell density and from that, algal behavior can be inferred. Both data sources together can be used to characterize algal bioreactor performance, using relevant characteristics such as oxygen evolution and carbon dioxide fixation rates per cell. The collected data increases the understanding of algal growth under reduced pressure and provides the basis for future overall system trade studies needed to implement an algal photobioreactor in a spacecraft. Even though primarily intended for conducting studies under reduced pressure, this setup can also be used to evaluate algae cultures under a variety of other conditions of interest for spaceflight applications, as well as for related terrestrial purposes.

4. **Demonstrated that altered atmospheric pressure within spacecraft relevant ranges as tested (at 8.2 and 14.7 psia) while maintaining normoxic conditions did not affect algal growth or metabolism.**

Performance of the algal culture was shown to be similar under the exploration atmosphere and standard sea level conditions both by cell growth (cell population density and optical density) as well as online metabolic measurements (cO_2, cCO_2, ΔpH, ΔDO). While a hypothesized increased performance could not be verified, it was shown that the nitrogen partial pressure does neither benefit nor limit the algal growth. The cause for this observation is thought to be the continuous mixture of the

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algal culture in this experiment that allowed diffusion to not be the limiting factor in algal growth.

5. **Carried out the first combination of metabolic measurements with metagenomic analysis of non-axenic cultures representative of spacecraft operational environments.**

The metagenomic analysis identified contamination of a 3-year culture representative of conditions in an operational laboratory environment such as a spacecraft. Such a combination of metabolic measurements with a metagenomic analysis has, to the best of our knowledge, not been performed before. Bacterial contamination was found leading to increased metabolic activity. Also, plant growth promoting bacteria were identified in the culture. Together with the existence of a variety of closely related algae from the family of Chlorellaceae that might have been in the algal culture from the manufacturer, the results allow a new angle for interpreting different algal metabolism studies. It also provides a cautionary example when interpreting results between strains of “the same” algal taxon.

6. **Conducted first-order feasibility analysis that established a minimum algal culture volume of 15 liters adequate to support one human in terms of air and water regeneration and derived the accompanying design requirements that were incorporated into a concept study.**

A successful first-order feasibility study of an algal photobioreactor for complete atmosphere revitalization for one crewmember was conducted. It was shown that an optimized algal photobioreactor of 15-liter culture volume could accommodate the air revitalization and urine processing needs of one crew member completely while also

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providing food supplementation. This number is lower than typically reported in the community. Based on the review of different studies it was concluded that photosynthetic rates are usually limited by the specific reactor implementation rather than by the biological potential itself. This analysis has developed key design requirements to overcome those limitations and has included them in a concept study that allowed the creation of a mass, power, and volume budget for the implementation of an algal photobioreactor into a spacecraft. Potential advantages over current ISS systems (comprised of OGA and UPA) for a crew of three in terms of mass and volume are shown. A potential disadvantage is the 5x increased power requirement, mainly for the LED lighting, when compared to non-algal systems for the sam functions. However, increased efficiency of oxygen recovery, water pre-processing, as well as positive effects on the food subsystem of an ECLSS, make further pursuit of this concept worthwhile to explore. The current flight-proven ISS systems CDRA and WPA are identified as supporting equipment that is continued to be needed with an algal life support system. Implementing an algal life support system into a spacecraft requires an optimized photobioreactor design in order to fully benefit from the biological potential of algae.

9.2 Synopsis of research objective outcomes

A total of four objectives were proposed in the comprehensive exam and outlined in Chapter 3.1. Additionally, two new objectives were added in the course of this work. Initially, a historical and internationally comprehensive list of algal flight experiments has been conducted that has shown limited results out of more than six

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decades of research. In parallel, a novel test bed was developed, characterized, and validated. The test reactor was successfully used to show that green algae are not dependent on the nitrogen partial pressure in the environment. Additionally, metagenomic analysis has provided valuable insight that allows a new angle for interpreting results from algal metabolism studies of the "same" taxon. A characterization of the effect of different oxygen concentrations at lower pressure was not performed, as the difference in concentrations between 38 % or 3.08 psia (sea level conditions) and 34 % or 2.78 psia (exploration atmosphere conditions) was not deemed significant enough. Lower partial pressures of oxygen are not realistic due to human physiology needs and higher concentrations would pose risks due to flammability concerns. While interesting from a phycology point of view, the effects of microgravity and chemical contaminants on algal behavior were deemed more important factors from a spacecraft implementation point of view. Using the results obtained in the previous objectives, a first-order feasibility study was conducted showing the biological potential of algae for life support applications. Both key design requirements as well as an implementation concept were derived that can be used for experimental verification as well as further analysis such as equivalent system mass.

As seen in Table 24, objectives 2, 3, and 6 have been met unconditionally. Two out of those three (objectives 2 and 6) have already been published in renowned conferences and journals with the third one (objective 3) being currently in preparation. Objective 4 was removed in the course of the dissertation as the significance was deemed minimal. Instead two new objectives (objective 1 and 5) were

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added in the course of the dissertation where objective 1 showed its relevance and quality by being invited to a journal publication.

Table 24: Status of research objectives

Obj.	Description	Status
1.	Assess the influence on microgravity on algal growth based on past flight-experiments.	✓
2.	Develop a novel test bed for flow-through measurements of algal metabolism under altered pressure for bioregenerative life support applications	✓
3.	Characterize the effect of varying total pressure on population growth and metabolism of *Chlorella vulgaris* at constant oxygen and carbon dioxide partial pressures	✓
4.	Characterize the effect of altered oxygen concentration on population growth and metabolism of *Chlorella vulgaris* under reduced total pressure	✗
5.	Characterize the effect of chemical contaminants on the health of algal cultures	✓
6.	Perform a first-order feasibility assessment of using an algal photobioreactor for spacecraft life support	✓

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9.3 Resultant publications and presentations

This dissertation resulted in a variety of publications covering a wide spectrum from technical journal publications over conference presentation to popular press articles for the general audience. Most of the publications are, in slightly modified form, included in this dissertation and marked appropriately after each section. A comprehensive overview of the publications and presentations directly related to this dissertation is presented below.

Journal Papers

1. **T. Niederwieser**, P. Kociolek and D. Klaus, "Spacecraft cabin environment effects on the growth and behavior of *Chlorella vulgaris* for life support applications", Life Sciences in Space Research, 16, pp. 8-17, 2018
2. **T. Niederwieser**, P. Kociolek, and D. Klaus, "A review of algal research in space," Acta Astronaut., 146, pp. 359–367, 2018.
3. **T. Niederwieser**, P. Kociolek, and D. Klaus, "Effect of altered nitrogen partial pressure on Chlorellaceae for spaceflight applications," Algal Research [IN PREPARATION]
4. **T. Niederwieser**, T. Ruck, D. Klaus, and A. Hoehn, "Feasibility study of an algal-based life support system," Acta Astronautica [IN PREPARATION]

Conference Papers (refereed)

1. **T. Niederwieser**, R. Wall, J. Nabity, and D. Klaus, "Development of a testbed for flow-through measurements of algal metabolism under altered pressure for bioregenerative life support applications;" in 47th Int. Conf. Environ. Syst. Charleston, SC, 2017 (ICES-2017-23)

Conference Papers (non-refereed)

1. **T. Niederwieser**, and D. Klaus, "Algal research in space," in Int. Astronaut. Congr. Adelaide, Australia, 2017 (IAC-17.A1.7.7x36885)
2. **T. Niederwieser**, and D. Klaus, "Feasibility study of an algal-based life support system," in Int. Astronaut. Congr. Bremen, Germany, 2018 (IAC-18.A1.7.9x43517)

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Posters

1. **T. Niederwieser**, "Recreating Earth in space: Spacecraft life support using algae;" in Austrian Res. Innov. Talk. Toronto, Canada, 2016.

2. **T. Niederwieser**, R. Wall, and D. Klaus, "Design consideration of an algal photobioreactor for implementation into a spacecraft environmental control and life support system;" in 7th Int. Conf. Algal Biomass, Biofuels, Bioprod. Miami, FL, 2017.

3. R. Wall, **T. Niederwieser**, D. Klaus, and J. Nabity, "Evaluation of agar-grown algae for environmental control and life support systems in spacecraft applications;" in 47th Int. Conf. Environ. Syst. Charleston, SC, 2017.

4. **T. Niederwieser**, "Optimization of algal photobioreactor concepts for implementation into spacecraft life support systems;" in 48th Int. Conf. Environ. Syst. Albuquerque, NM, 2018.

Presentations

1. 47th International Conference on Environmental Systems (ICES), "Development of a testbed for flow-through measurements of algal metabolism under altered pressure for bioregenerative life support applications" Charleston, SC, 2017.

2. 68th International Astronautical Congress (IAC), "Algal research in space" Adelaide, Australia, 2017.

3. NASA Deep Space Gateway Science Workshop, "Basic and applied algal life support system research on board the Deep Space Gateway" Denver, CO, 2018.

4. 69th International Astronautical Congress (IAC), "Feasibility study of an algal-based life support system" Bremen, Germany, 2018.

Popular Press

1. **T. Niederwieser**, "Using algae to support astronauts on deep space missions", ROOM – The Space Journal, 16(2), pp. 45-49, 2018.

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9.4 Resultant outreach component

This dissertation resulted in the direct involvement of several students in the planning, execution, and analysis of experiments. Students ranged from undergraduates to graduates and over periods ranging from weeks to a full year as outlined below.

Undergraduate Involvement

1. University of Colorado Boulder, Undergraduate Research Opportunities Program (UROP), Ryan Wall, May 2016 to August 2017
2. The BOLD Center, Spring Break for Research, Emily Weidenfeller, March 2018

Graduate Involvement

1. Technical University Munich, Master's Thesis, Thomas Ruck, July 2018 to November 2018

9.5 Future work

In summary, this thesis enhanced both the biological understanding of algal metabolism as well as the technical understanding of implementing an algal photobioreactor into a spacecraft life support system from the engineering perspective. Algal life support systems pose an intriguing possibility for future bioregenerative life support systems on long duration spaceflights. This is especially true for water recycling, air revitalization, and food supplementation. However, in order to make algal photobioreactors a reality, it is crucial to demonstrate key aspects such as long-term stability, system robustness, interactions with other systems in a closed loop, and ease of operability. First, the adaptation of rapidly growing algae to the space environment, namely radiation and microgravity, has to be understood. Secondly, it is of crucial importance to demonstrate an algal life support system in a

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spacecraft showing robustness and operability under low risk in microgravity conditions and the deep space radiation environment while still being in close proximity to Earth to iterate or fine tune the design before eventually using a mature system on a long-term mission. The International Space Station or Lunar Gateway are suitable platforms as they allow a test in a real mission environment. From a small-scale laboratory demonstration unit, the technology can be optimized, and the assumptions verified. Initially, a one-person scale is suitable for laboratory tests, but ultimately the technology has to prove itself in a full-scale flight demonstration.

Lastly, we have to find a way to solve the challenge of making algal food supplement appetizing for humans. At the same time, algae offer unique potentials such as high reliability and psychological benefits for the crew if the stereotypes against biological systems are overcome. Through new innovative designs, biology and engineering can exist in symbiosis that benefit both sides. Additionally, while this technology development is tailored for spaceflight life support systems that might one day allow us to travel to and live on Mars and beyond, it might also help us to make life on Earth more sustainable by reducing the carbon dioxide concentration.

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