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Evaluation of a Flow-Through Test Bed for Algal Atmosphere Revitalization in Spaceflight Applications

Tobias Niederwieser
University of Colorado at Boulder, toni9434@colorado.edu

EVALUATION OF A FLOW-THROUGH TEST BED FOR ALGAL ATMOSPHERE REVITALIZATION IN SPACEFLIGHT APPLICATIONS

by

TOBIAS NIEDERWIESER

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Advisor: Prof. David Klaus

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Prof. David Klaus

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Prof. James Nabity

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Evaluation of a flow-through test bed for algal atmosphere revitalization in spaceflight applications

Thesis directed by Associate Professor David Klaus

Air revitalization is a crucial element in human spaceflight. Current technology is consumable based and only suitable for short duration missions. A potential candidate for long duration spaceflight missions or planetary habitats is algae, which can be used to reduce carbon dioxide to oxygen. Previous work has been performed in characterizing the biological principles of algae regarding their adaptation to different environmental parameters. However, in order to integrate algae into a spacecraft air revitalization system further data is needed. One approach to collect these data is using a flow-through set up that measures input and output gas compositions, which can then be used to determine time dependent adsorption and desorption rates. For algae, however, tests using this approach were not found in the literature. This thesis makes an attempt to get a step closer to enabling an algal flow-through experimental set up. It also shows the challenges that are associated with measuring gas compositions in algal systems, as their oxygen production rate is only 200 fmol/(cell•h). This requires sensor resolutions smaller than 100 ppm and flow rates as low as 10 ml/min. Due to permeability of carbon dioxide through tubing and the solubility of carbon dioxide in water the feasibility could not be proven with a demonstration unit. However, environmental parameters for optimum growth and design recommendations are summarized that will overcome the difficulties and support future flow-through experiments.

I. Acknowledgments

I want to thank my advisor Professor David Klaus and the committee members Professor James Nabity and Professor Louis Stodieck. This thesis would have not been possible without the great financial support through the William F. Marlar Memorial Foundation and support from the LifeLAB Graduate Projects Team that prepared AETHER for these tests and allowed me to block valuable testing time. Additionally I would like to honor the efforts by BioServe Space Technologies for allowing me the use of their facilities.

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III. Abbreviations

AETHER	Atmospheric and Environmental Test Hub for Experimentation on Revitalization
BLSS	Bioregenerative Life Support System
CELSS	Controlled Ecological Life Support System
DAQ	Data Acquisition
ECLSS	Environmental Control and Life Support System
FEP	Fluorinated ethylene propylene (Teflon)
LED	Light Emitting Diode
MELiSSA	Micro-Ecological Life Support System Alternative
NASA	National Aeronautics and Space Administration
NPT	National Pipe Thread
TRL	Technology Readiness Level

IV. Variables

m	Mass	$[kg]$
$\dot{m}$	Mass flow rate	$[\frac{kg}{s}]$
n_{cells}	Number of cells	$[-]$
n_{O_2}	Molar amount of oxygen	$[mol]$
$\dot{n}_{O_2}$	Rate of oxygen production	$[\frac{mol}{s}]$
p	Pressure	$[kPa]$
R	Gas constant (8.314)	$[\frac{J}{mol \cdot K}]$
T	Temperature	$[K]$
V	Volume	$[m^3]$
$\dot{V}$	Volumetric flow rate	$[\frac{l}{min}]$

1. Introduction

Space is a very harsh environment that induces threats to life. In order to support human life in space, scientists and engineers have developed technologies that provides human beings a habitable environment. Aside from providing temperature control, water recycling, waste removal and food provision in this environment, revitalization of cabin air is an essential element. Without any kind of air revitalization, the oxygen concentration in a sealed capsule would decrease. At the same time the carbon dioxide levels would increase to life threatening levels due to the metabolic rates of human beings as shown in Figure 1 (Larson and Pranke, 1999).

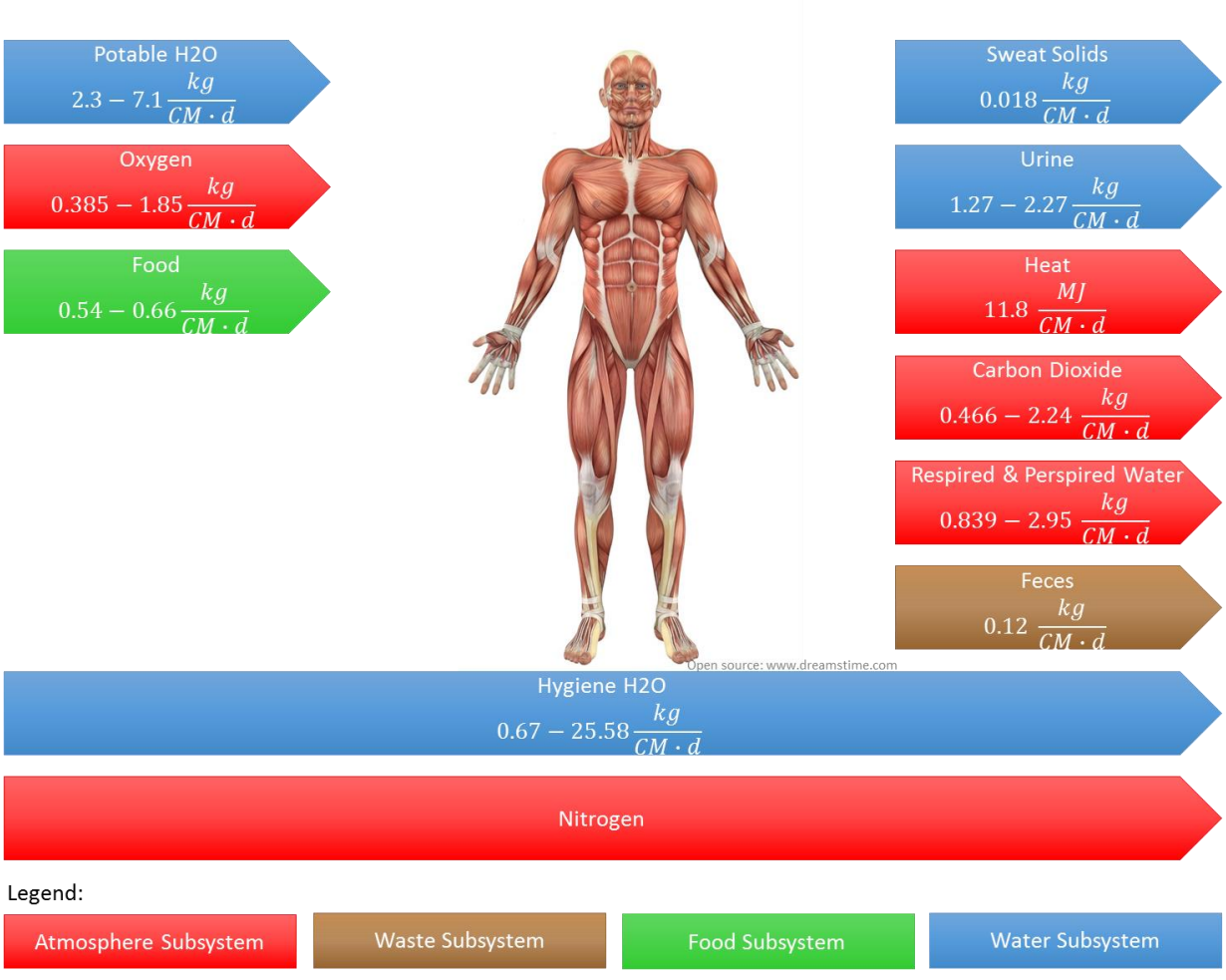

Figure 1: Human metabolic rates divided into different subsystems

In the past and current vehicles, physicochemical life support systems have been used to maintain a habitable atmosphere. In this specific type of environmental control and life support system (ECLSS) man is the only biological component (Eckart, 1996). All other functions were either performed by chemicals such as lithium hydroxide for removing carbon dioxide, or by consumables, such as oxygen from supply tanks. However, every single mission in the past was either short term or in close proximity to the Earth, allowing for a continuous consumables resupply chain (Wieland, 1998). According to NASA's exploration roadmap, the goal is to pursue long duration missions to the Moon, Mars or asteroids (National Research Council, 2014). These future missions would last 300 days and longer, and would not be resupplied on a frequent basis. Additionally, taking all the necessary consumables on the travel would make a spacecraft too heavy to launch, which established the need for new technologies. At the moment research is being done on regenerative life support systems, which recycle the carbon dioxide back to oxygen. NASA identifies this in the Space Technology Roadmaps Technology Area 06 "Human Health, Life Support & Habitation Systems" (Hurlbert et al., 2012).

As indicated in Figure 2, one approach to recycle oxygen within a spacecraft is to use physicochemical life support systems. To date all missions have used this path with technologies such as molecular sieves, solid amine water desorption or electrochemical depolarization concentrators that are used to concentrate carbon dioxide. This stream can then be fed into a Bosch or Sabatier reactor or another system to reduce the carbon dioxide to methane and oxygen (Eckart, 1996). Physicochemical life support systems can also be used for water recycling. However, they cannot be used for closing the carbon loop within a spacecraft, as food cannot be produced. Additionally, physicochemical systems are highly complex and have a very low meantime between failure.

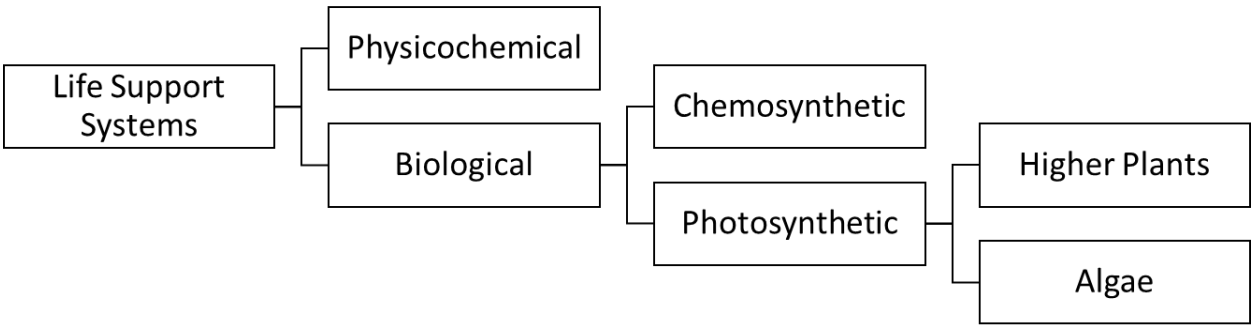

Figure 2: Classification of life support systems

The other approach is to develop biological life support systems. As these systems are able to produce edible biomass it is possible to close the oxygen, water and carbon loop within a spacecraft. These systems convert carbon, usually found in carbon dioxide, into biomass. Theoretically they are hence only limited by leakage of materials out of the habitat. Two main approaches are used. In a chemosynthetic system this process gains the energy from chemical reactions whereas photosynthetic systems use light as the energy source. Within photosynthetic systems either higher plants or algae can be used. Reasons why research is also focused on algae include a smaller system volume as well as a more suitable assimilatory quotient to humans. Since algae are performing the following reaction to reduce carbon dioxide the assimilatory quotient can be calculated to 0.89 (Eckart, 1996):

$$6.19\ CO_2 + 3.7\ H_2O + NH_3 + light \rightarrow\ C_{6.14}H_{10.3}O_{2.24}N + 6.9\ O_2$$

This is closer to the average human respiratory quotient of 0.8 than higher plants. One concept of implementing an algal photobioreactor is the water wall concept, which is developed

at NASA Ames Research Center. This concept claims to incorporate an entire biological life support system in the shell of the spacecraft. By mounting five layers of 1 liter bags to the entire inner surface of the shell in a honeycomb pattern, it consumes less volume than current physicochemical systems, acts as radiation protection and can help improve the psychological well-being of the crew. It uses five different kinds of bags that each fulfill a different life support function among which one is air revitalization. Its main advantage is the increase in reliability to current life support systems. Due to multiple bags fulfilling one function a failure of one single bag does decrease the efficiency but keeps the entire system running. Also, maintenance can be performed by switching out a bag in a running system. If used with biological cultures, this approach confines the spread of disease to just one bag and thereby prevents infection of the entire culture. The air revitalization bags will be filled with an algal solution, which is thermally controlled to room temperature of 22 °C with an external water loop. No external lighting is provided and hence the lighting from the cabin is used for the photosynthetic activity. Since the bags are mounted to the spacecraft's shell, only one side of the bags is illuminated. In order to provide illumination of the entire bag a thickness of the inflated water bag of only 2.5 cm was chosen. Due to a gas permeable membrane carbon dioxide as well as oxygen is able to pass through the wall whereas water is not able to pass. Since algae consumes carbon dioxide and produces oxygen, a concentration difference will be established between the cabin air and the water bag, forcing these gases to diffuse through the membrane. Tests with blue-green algae have resulted in a total carbon dioxide fixation between 50 and 250 mg/(l•h) dependent on the species (Cohen et al., 2013). The negative effect of blue-green algae is that they are not edible for the crew. Therefore, future tests with the green algae chlorella are planned (Cohen et al., 2014).

Another attempt to recreate a biosphere within a spacecraft is performed by the Micro-Ecological Life Support System Alternative (MELiSSA) Project (Hendrickx et al., 2006). Within four steps it is tried to balance the human inputs and outputs. Waste is treated with bacteria to decompose it to carbon dioxide and nitrates. Together with the carbon dioxide produced by the crew metabolism, carbon dioxide and nitrates are converted to oxygen and food by two different means. The first process uses higher plants to grow both food and oxygen whereas the second process utilizes the cyanobacteria Arthrospira spirulina. Different from other cyanobacteria, arthrospira is also edible and at the same time has a very high production rate, while being easy to culture. Apart from oxygen and food it also produces water out of hydrogen and nitrate. An advantage of having a combination of higher plants and algae is increased reliability due to two unequal systems. In case parasites attack an algal culture and spread unhindered in the monoculture the higher plant compartment can still function separately. By only having a monoculture it will take weeks to regrow the required cell count to support a crew.

2. Background

Algae in general can be divided into two different categories. Cyanobacteria, which are often referred to as blue-green algae, are bacteria without nucleus (prokaryotic). The second category are green algae, which are cells with nucleus (eukaryotic). In selecting the appropriate algae a green algae strain was chosen over a blue-green algae strain as their biomass is edible. This not just reduces waste but also complements the main source of food provision with fresh food. Generally speaking cyanobacterial blooms on the other hand are often toxic even though some edible species exist. Different algae strains were researched in the past regarding their doubling time which ranges from as low as 141.2 hours to as high as 2.1 hours (Hoogenhout and Amesz, 1965). Due to ease of culture and an extensive availability of literature, chlorella vulgaris was chosen as the species in this thesis (Kammermeyer, 1966). On the other hand chlorella vulgaris also provides a high doubling time of 9 hours. The source of chlorella vulgaris in bacteria free form was found at Carolina Biological Supplies.

2.1. Environmental factors affecting the growth of chlorella vulgaris

In order to evaluate the feasibility of algal test beds it is important to know the effects of environmental parameters on the growth behavior as they have to be accommodated in the test set up. Hence a literature search was conducted. Below is a list of research performed on the effects of environmental parameters on chlorella algae.

2.1.1. Carbon dioxide concentration

Chlorella vulgaris was researched between ambient levels (0.038 %) and 20 % of carbon dioxide. This was accomplished by a continuous stream of carbon dioxide enriched air at temperatures of 30, 40 and 50 °C. From ambient levels to about 6 % the biomass increased with

higher levels of carbon dioxide. This is followed by a plateau of biomass production between 6 and 10 % before it drops to below ambient levels with increasing carbon dioxide levels. The optimum for biomass production was therefore concluded to be at 6 % carbon dioxide concentration (Chinnasamy et al., 2009).

Another investigation on the effect of elevated carbon dioxide levels showed an optimum in biomass production at 4 % (Bhola et al., 2011; Singh and Singh, 2014). Surprisingly this study was also conducted between ambient levels and 15% of carbon dioxide but at 25 °C. The carbon dioxide however was controlled once a day to the desired set point. Due to the fact that the study performed by Chinnasamy et al. (2009) has a more steady control of the carbon dioxide level and better data of the algal growth at different temperatures their paper was chosen as reasoning for selecting a carbon dioxide concentration of 6 %.

2.1.2. Oxygen concentration

A high partial pressure of oxygen can cause oxygen toxicity in algal cells and is referred to as the Warburg effect. Higher oxygen partial pressures than 21 kPa start decreasing the photosynthetic efficiency and pressures above 93 kPa have a 75 % decrease of growth rate (Kammermeyer, 1966; Myers et al., 1961). Algae however can work in an environment with highly reduced oxygen partial pressure (Chinnasamy et al., 2009).

2.1.3. Temperature

Previous research was conducted to determine the effect of temperature on the growth of chlorella vulgaris. The growth was measured at 30, 40 and 50 °C. Highest biomass production was seen at 30 °C even at different carbon dioxide concentrations of 0.036 and 6 % (Chinnasamy et al., 2009).

2.1.4. Light intensity

Different species of green algae were researched under different levels of light intensity between 0 and 107000 lx. All of the green algae show an almost linear rise in algal growth with an increase in light intensity while the carbon dioxide concentration was held constant at 4 %. Once the saturation point is reached between 2700 lx and 6500 lx the algal growth stays constant even with increasing light intensities. Once the light intensity is increased to above 22000 lx the algal growth is suddenly inhibited in all species. Since there is no need to go higher than 2700 lx this is the required value for the test set up (Sorokin and Krauss, 1958).

2.1.5. Light cycle

Close to all studies performed used a light cycle of either 12/12 (Chinnasamy et al., 2009; Scarsella et al., 2010; Wang et al., 2004), 14/10 (Cuaresma et al., 2011) or 16/8 hours (Cohen et al., 2013). A novel approach is using a strobe light effect for increasing the photosynthetic efficiency of chlorella. A study investigating light cycles from 2-100 Hz found a 21 % increase in biomass production at 100 Hz (Liao et al., 2014).

This increase is thought to be explained due to biological processes within the algae cell. After long exposures to oversaturated levels of light photoinhibition due to oxygen radicals in photosystem II can occur. (Long et al., 1994; Nixon et al., 2010). This inhibits the electron transport needed to reduce water into oxygen and hydrogen ions.

2.1.6. Light spectrum

Chlorella cells absorb light at wavelengths between 300 to 700 nm and have their maximum absorptivity in the red (450 nm) and blue (650 nm) regions (Kammermeyer, 1966; Myers and French, 1960; Schettini, 2004). This also conforms with other studies conducted on chlorella vulgaris algae that used peaks at 680 nm (Lee and Palsson, 1994; Liao et al., 2014).

2.1.7. Gravity

Several space missions with algae have been flown in microgravity to date. Popova and Sytnik (1996) launched chlorella cells on the biosatellite Bion-10. Important findings were a 112 % increase in mitochondria volume due to an increased mitochondria size as well as a decrease of starch volume to 26 % of the ground control (Popova and Sytnik, 1996). A very important side note on that experiment is that all data were collected post flight. With that a potential re-adaptation to gravity could already have occurred before fixation. Wang et al. (2004) however did a study on the cyanobacteria Nostoc sphaeroides Kuetz. Their increase in biomass in space was 150 %, whereas their increase on ground was just 18 % from the start of the experiment (Wang et al., 2004). Nevertheless, in their data from their space samples they observed some sudden spikes and fluctuations in population density that could indicate a reduced quality of the measuring method in space.

Additionally a novel approach was chosen in a study performed on ground that compared horizontal vs. vertical oriented photobioreactors (Cuaresma et al., 2011). While being a potential indicator for gravity dependence of the growth of algal cells, the way the experiment was conducted does not allow any conclusions about the size of the base surface area on the algal growth. While holding environmental parameters constant, the light intensity was varied during the day as the orientation of the reactor changes the normal surface area facing the sun. The actual reactor however was never rotated. The simulated vertically placed photobioreactor showed a 50 % higher photosynthetic efficiency compared to the horizontal case.

Comparing all these studies it becomes clear that the effect of gravity on the growth of algal cells needs more study.

2.1.8. Summary

As seen within sections 2.1.1 to 2.1.7, there are a factor of environmental parameters that influence the growth rate of chlorella vulgaris cells. For a life support system that is mass and volume efficient it is important to hold the environmental factors at the optimum. A summary of the researched values is presented in Table 1.

Table 1: Summary of environmental parameters for optimal growth rates of chlorella vulgaris

Parameter	Value	Reference
Carbon dioxide	4, 6 %	(Bhola et al., 2011; Chinnasamy et al., 2009; Singh and Singh, 2014)
Oxygen	<21 kPa	(Chinnasamy et al., 2009; Kammermeyer, 1966; Myers et al., 1961)
Temperature	30 °C	(Chinnasamy et al., 2009)
Light		
Intensity	2700 lx	(Sorokin and Krauss, 1958)
Cycle	12/12, 14/10, 16/8 hours 100Hz	(Chinnasamy et al., 2009; Cohen et al., 2013; Cuaresma et al., 2011; Scarsella et al., 2010; Wang et al., 2004) (Liao et al., 2014)
Spectrum	300-700 nm	(Kammermeyer, 1966; Myers and French, 1960; Schettini, 2004)
Gravity	Both higher and lower growth rates observed	(Vasilenko and Popova, 1996; Wang et al., 2004)

Due to the variety of parameters that are also usually provided in a range, the values of carbon dioxide reduction and growth rates between different studies differ a lot. This problem is very well summarized in the following quote (Lembi and Waaland, 1990, p. 494):

"A review by Miller and Ward (1966) of numerous algal growth systems showed that the requirements of an algal reactor to provide gas exchange for one human varied from 3.5 to 3,000 liters of algal culture, from 2.5 to 71 m^2 reactor surface area, and from 7 to 100 kW electrical power."

This shows that there is the need to perform further research on parameters like gravitational influences as well as applying the earlier findings on environmental effects into one system to show the feasibility of this technology.

2.2. Experimental set up

In Table 2 an overview of research on algal characterization that has been performed is given. This list provides a brief overview on the methods of how growth rate of algae is measured and how the experiments are conducted. By reviewing the literature the test set ups were categorized in the following three methodologies - batch, recirculated flow and flow-through cultures as seen in Figure 3. In a batch culture the algal solution is within an encapsulated chamber. At the beginning the chamber is incubated to the desired gas composition but during the test there is no material flow through the borders of the chamber. A recirculated flow resembles the batch culture. The flow is moving through the algal solution at a certain flowrate. The outlet most often is directly connected to the inlet via a gas analyzer. Some studies add gases to the recirculated flow at a very slow rate so the composition inside the set up stays constant. This technically is not pure recirculated flow but more a mix with the third category which is the flow-through set up.

Table 2: Characterization of set up and measurement type of previous research performed on chlorella algae

	Batch	Recirculated flow	Flow-through
Cell count	(Popova, 2003; Vasilenko and Popova, 1996; Wang et al., 2004)	(Lee and Palsson, 1994; Smernoff, 1986)	(Sakai et al., 1995; Sorokin and Krauss, 1958)
Biomass	(Cohen et al., 2013; Metaferia, 2014)	(Cuaresma et al., 2011; Lee and Palsson, 1994; Smernoff, 1986)	(Chinnasamy et al., 2009; Hoogenhout and Amesz, 1965; Scarsella et al., 2010; Yeh et al., 2010)
Gas analysis	(Bovee et al., 1962; Doney and Myers, 1958; Kirensky et al., 1968, 1967)	(Cuaresma et al., 2011; Lee and Palsson, 1994; Smernoff, 1986)	-

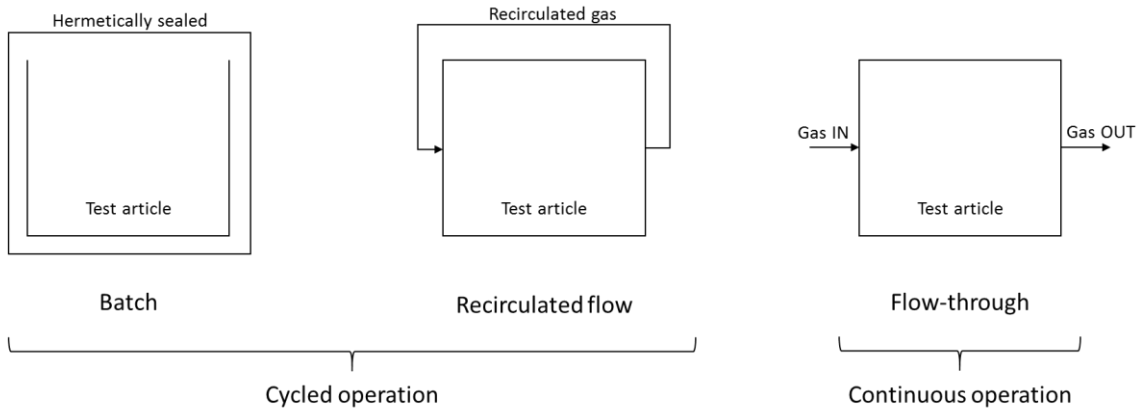

Figure 3: Variations on gas flows used in different test set ups

The flow-through set up is a very common way to characterize low technology readiness level (TRL) ECLSS technologies. Hereby a precise gas composition is fed into the chamber at a certain flowrate. At the outlet the air stream is thoroughly characterized before it is vented. By comparing the air inlet and outlet compositions it allows a means to measure the total amount of desorption or adsorption as well as the chronological sequence of events. Surprisingly, these flow-through experiments have not yet been performed on algae.

To date the most common way is to record the algal mass by drying the algal solution around 80 °C and weighing the remaining dry biomass (Chinnasamy et al., 2009; Cohen et al., 2013; Hoogenhout and Amesz, 1965; Metaferia, 2014; Scarsella et al., 2010; Yeh et al., 2010). There are factors available to convert biomass to the amount of oxygen produced or carbon dioxide reduced (Kammermeyer, 1966). This is the reason why a lot of research groups also specify a number of total carbon dioxide removed even though it was not specifically measured. Another very common method is measuring the cell count, which is mainly performed in three different ways. First, a sample can be counted either by hand or by software under a microscope (Popova and Sytnik, 1996; Popova, 2003). From this value the total population density can be applied to the entire solution volume to gain the cell count. Secondly, a spectrophotometer can be used to measure the absorptivity of the solution (Sakai et al., 1995; Sorokin and Krauss, 1958; Wang et al., 2004). More cells will cause more shadowing. From reference measurements these absorptivity values can then be mapped to a population density and cell count. The third measurement method found is measuring the carbon dioxide or oxygen concentration in the gas. However there were only three studies that conducted this type of measurement (Cuaresma et al., 2011; Lee and Palsson, 1994; Smernoff, 1986).

2.2.1. Flow-through

Other technologies on air revitalization have been previously evaluated in a flow-through set up. This approach was for example chosen to quantify adsorbing and desorbing rates of carbon dioxide and water in potassium dioxide (Holquist et al., 2013). The initial attempt to measure a flow-through set up was performed with small scale test-tube set up, which can be seen in Figure 4. With the help of flow meters (2) and a humidifier tank (1) a certain concentration of carbon dioxide (0.5 %), flowrate (74 ml/min) and relative humidity (53 %) was established and measured

with sensors (4) at the beginning of the test. After the gas provision reached steady state, the test article (3) was put in line and the sensors (4) were used to solely measure the output of the test tube and record it on the computer (5). The input was assumed to stay constant over the test duration.

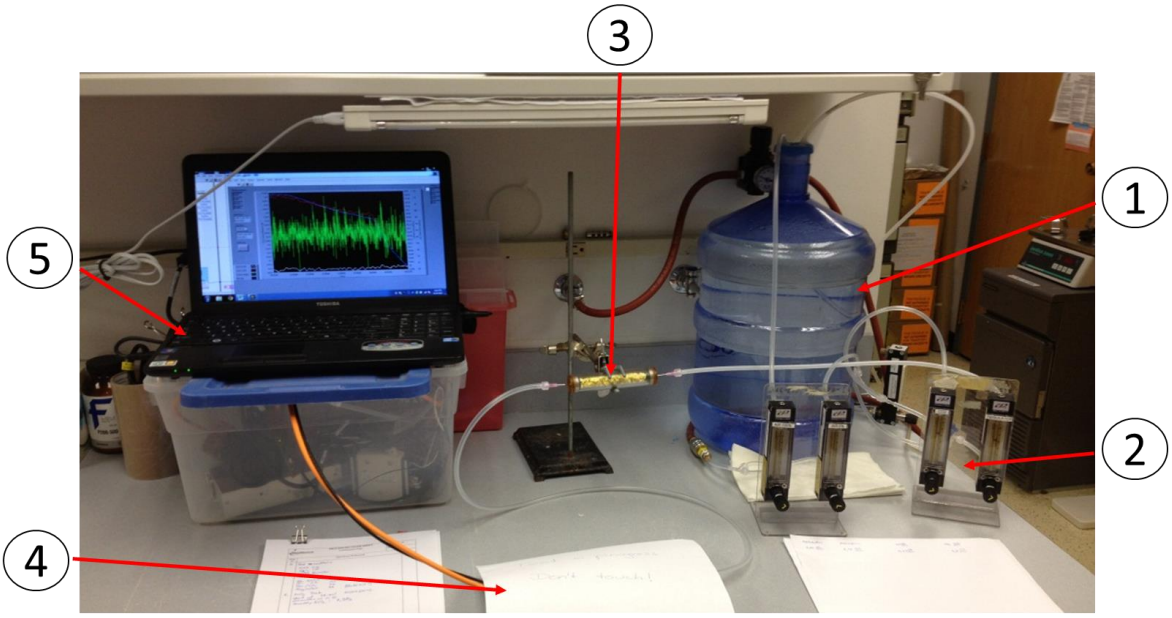

Figure 4: Small scale flow-through set up (1 humidifier; 2 flowmeter; 3 test article; 4 outlet stream sensors; 5 DAQ computer)

This system was helpful to understand the working principle of potassium dioxide but did not satisfy the very precise measurement requirements. The biggest problems were an insufficient flow rate for bigger diameter test tubes as well as the realization that the input air stream did not stay constant over time. It varied especially in relative humidity due to slightly different room temperatures during a night/day cycle and in flow rate due to decreasing supply pressure in the gas cylinders. For this reason a test chamber (4) was built that holds a set of sensors on both the inlet (3) and outlet (5) of the test article, which allows more precise measurements (Figure 5). Furthermore it accommodates bigger diameter tubing to allow higher flow rates of approximately 700 ml/min.

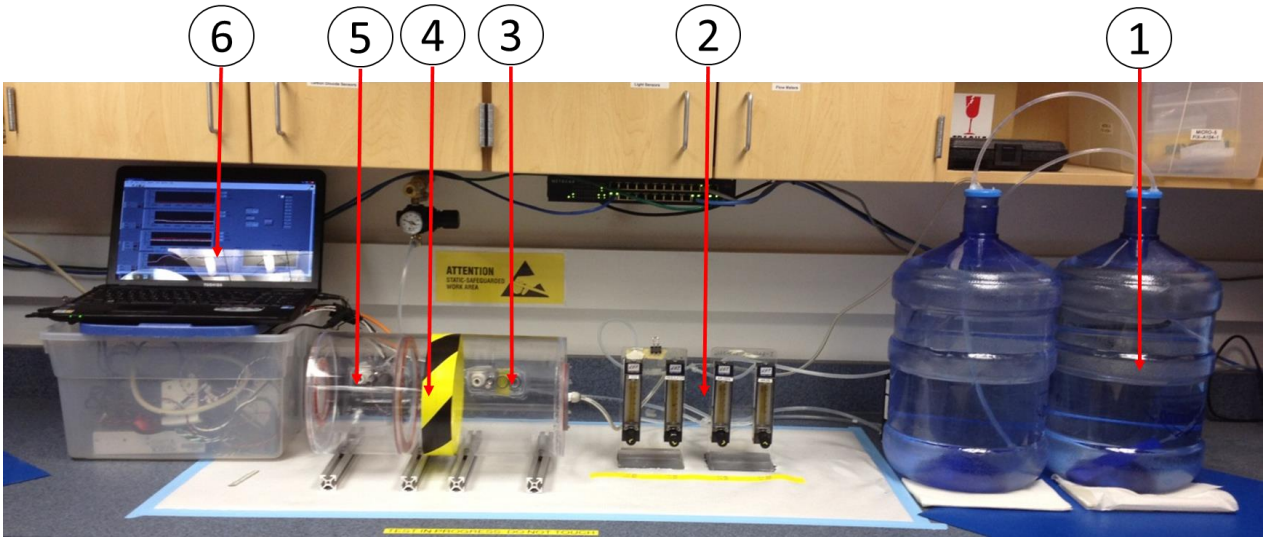

Figure 5: Large scale flow-through set up (1 humidifier; 2 flowmeter; 3 inlet sensors; 4 test article; 5 outlet sensors; 6 DAQ computer)

For future research it was decided to develop a more advanced facility to perform these kind of flow-through tests. Major improvements were automated flow control to gain a more constant gas composition over the test duration of usually a couple of hours to days. Also the humidifier was redesigned to gain higher humidity levels. Additionally a more permanent sensor placement was desired to gain a better comparability between tests that are performed within a time span of several months. This lead to a universal test bed called the Atmospheric and Environmental Test Hub for Experimentation on Revitalization (AETHER) located in the bioastronautics facility of the University of Colorado Boulder (Figure 6). It is currently designed to provide a flow rate of any composition of shop air, carbon dioxide, oxygen and nitrogen between 2-10 slpm. In addition there is a port to insert an additional experiment specific gas into the inlet stream. Other improvements that AETHER accommodates are an increased height of the water column (2) that the air stream is bubbling through to achieve a higher relative humidity. Besides, the outlet tubing on the humidifier was increased from 1/8 inch flex tubing to 3/4 inch pipe to decrease pressure effects on the air stream that influence the relative humidity level. Furthermore

AETHER provides a set of sensors at the inlet (3) and outlet (6) of the test article as well as a bypass stream (5) to validate measurements or initialize the flow. Even though this is a permanent installation the test chamber (4) is completely exchangeable, making it modular to different experimental set ups.

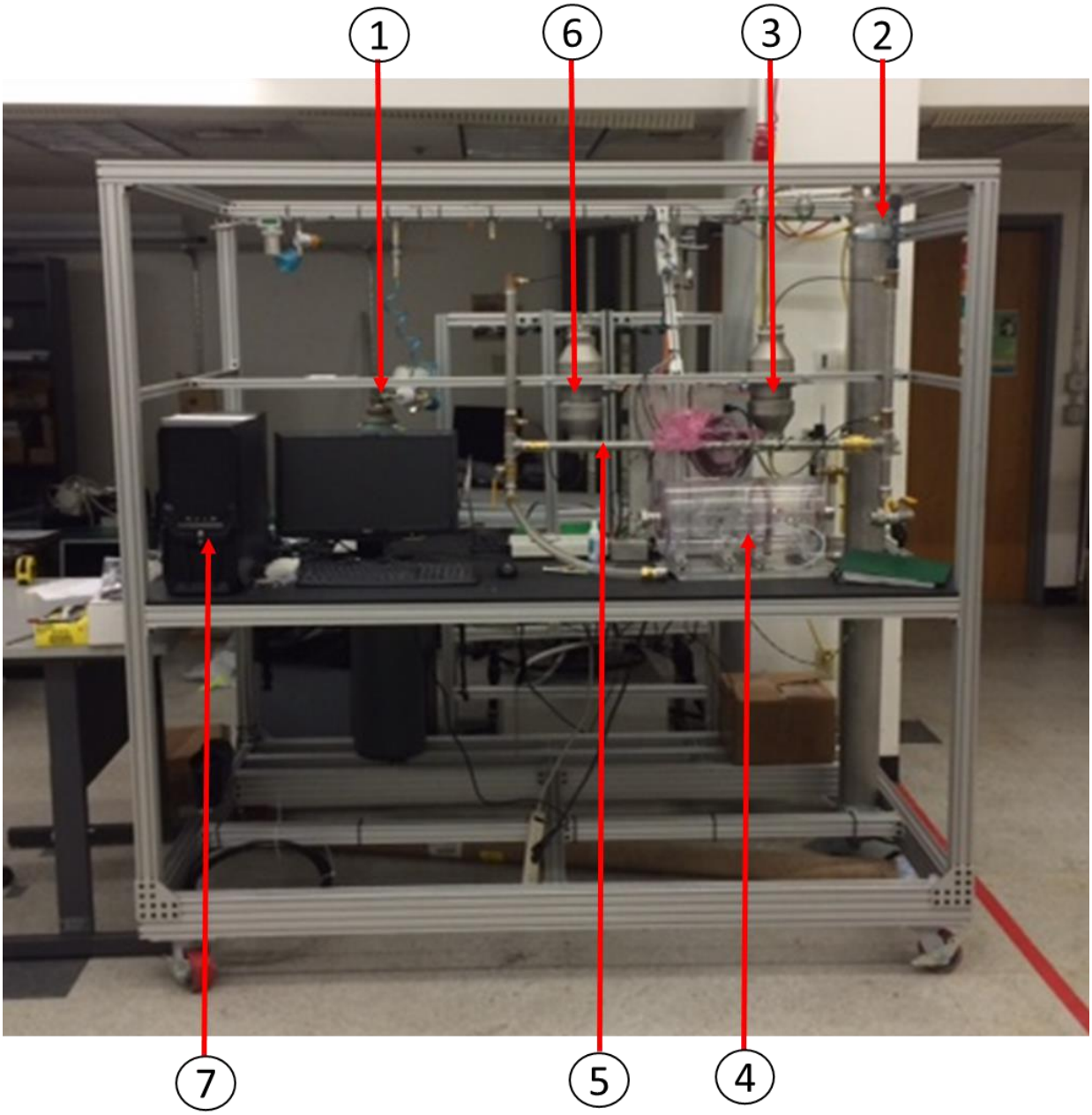

Figure 6: AETHER test facility for flow-through tests (1 gas cylinders; 2 humidifier; 3 inlet sensors; 4 test chamber; 5 bypass; 6 outlet sensors; 7 DAQ computer)

3. Problem statement

As laid out in section 2.2, the literature has shown that there have been a variety of studies on algae in a closed chamber. Mostly the interest was in the effects of environmental parameters on the produced biomass for fuel or food production. The chosen set up for these kind of studies ranges from batch processing to flow-through, while measuring biomass at the end. Very few papers actually looked at the oxygen production and carbon dioxide reduction rate. The ones that did exclusively looked at recirculated flow instead of flow-through. On the other hand there have been several flow-through studies on air revitalization technologies to characterize the performance. In order to compare algal air revitalization to competing systems it would be valuable to characterize that in flow-through systems. For this the following task was developed:

Evaluate the feasibility of utilizing a flow-through testing facility for measuring carbon dioxide reduction and oxygen production rates of algae.

4. Pilot study

In order to design the system a potential experiment was developed. As concluded in section 2.1.7 the effects of gravity on algae cells are not fully understood. Since algae cells stick to surfaces and settle on the floor in a gravitational environment, the mixing type is a very suitable independent variable and can be used as an example in this set up. With that the following potential research hypothesis was established:

Stirring increases the oxygen production, carbon dioxide reduction and biomass production of chlorella vulgaris at the same effect as sparging relative to an unstirred, batch baseline.

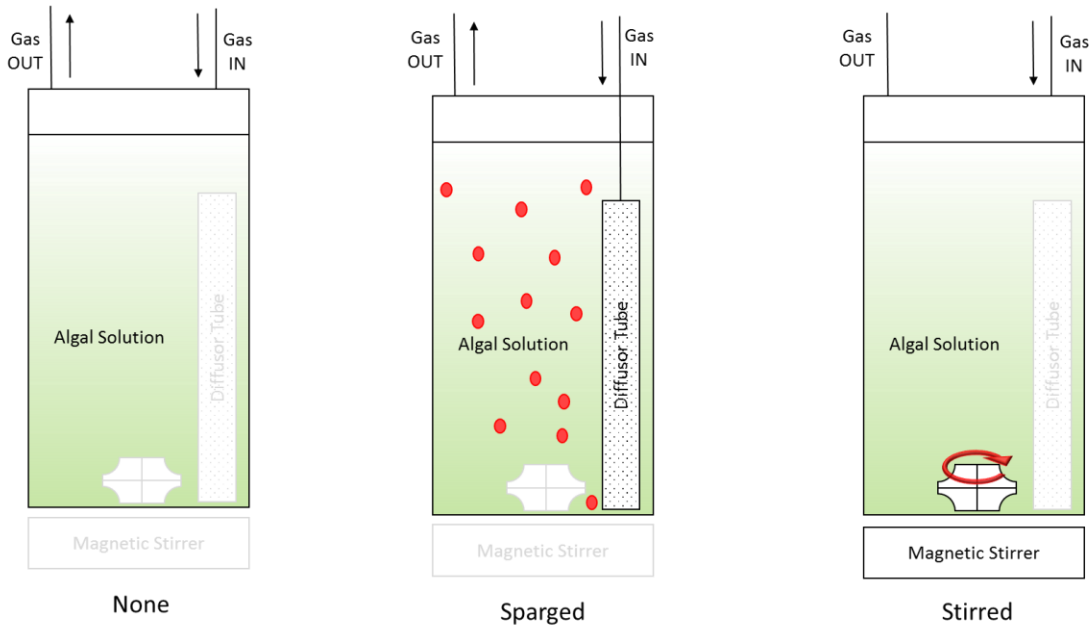

Figure 7: Schematic view of various algae growth methods (none, stirred and sparged)

Figure 7 shows the difference between the different mixing methods. First option is no mixing at all as a reference case. Secondly, the stirred case uses a magnetic stirrer to create a vortex

within the reaction chamber. Lastly, the sparged case uses the raising gas stream bubbles to create turbulences within the chamber and therefore mixing. The latter is the most common system to cultivate algae on ground. However, due to the lack of gravity this system is not feasible in space. For developing the set up under realistic conditions the following variables got defined as seen in Table 3 with the help of the literature search conducted in chapter 2. It is important to note that the surface within the algal solution has to remain constant. For that reason the diffuser tube is placed in all set ups even though it is in use in only one.

Table 3: Definitions of test variables

Independent variable:	Mixing Type (None, sparging, stirred)	
Dependent variables:	Oxygen production rate	
	Carbon dioxide reduction rate	
	Biomass production	
Confounding variables	Shadowing	
Constant variables:	Initial algal density	($5 \cdot 10^5$ cells/ml)
	Temperature	(30 °C)
	Initial Carbon Dioxide concentration	(6 %)
	Light intensity	(810 lumens)
	Light cycle	(12/12)
	Light spectrum	(cool white 6000K)
	Volume of algal solution	(1 L)
	Volume of gas	(const.)
	Surface area of algal solution	(const.)

5. Materials and methods

5.1. Calculations

In order to use AETHER as the test facility preliminary calculations were made to predict the expected measurements.

Table 4: Assumptions for algae production in a flow-through test

Variable	Value
Algal solution volume	$4\ l$
Average population density	$10^6\ \frac{cells}{ml}$
Oxygen production	$200\ \frac{fmol\ O_2}{cell \cdot h}$
Flow rate	$1\ slpm$
Temperature	$30\ °C$
Pressure	$\sim 81\ kPa$

The rate of carbon dioxide reduction assuming a 4 liters algae bag can be calculated to

$$\dot{m} = 4\ l * 0.0768\ \frac{g}{l \cdot d} = 0.3144\ \frac{g}{d}$$

This correlates to a volumetric reduction rate of

$$\dot{V} = \frac{0.3144\ \frac{g}{d}}{1.842\ \frac{g}{cm^3}} = 0.171\ \frac{l}{d} = 0.1\ \frac{ml}{min}$$

At a flow rate of 1 slpm at 6 % carbon dioxide level the flow rate of carbon dioxide is 0.06 slpm. A reduction at the rate of 0.1 ml/min hence would decrease the carbon dioxide concentration from 6.00 % to 5.99 %. This is within the uncertainty of the sensor and therefore cannot be used to distinguish two different reduction processes, especially since the interest lays

in the slightly higher or lower absorption rates which makes it even harder to distinguish. Solving this issue would involve increasing the volume of the algal solution to about 400 liters or decreasing the flow rate to about 10 ml/min in order to get a high enough resolution. Of course any combination thereof is also possible. However, getting precise measurements at these low flow rates as well as providing a 400 liters sealed gas tank is challenging. Most algae studies are conducted in lab set ups in up to 4 liter containers. Cultures will behave differently in big cultures due to shadowing effects in the solution. In order to sustain comparability to other studies it was decided not to increase the chamber size.

The easiest method to increase the difference in carbon dioxide concentrations would be a change from a flow-through set up to a recirculated flow. With the help of time the initial difference over time increases until it is measurable by the sensors. The feasibility was then shown by calculations with the assumptions presented in Table 5.

Table 5: Assumptions for algae production in recirculated flow tests

Variable	Value
Algal solution volume	$1\ l$
Average population density	$10^6 \frac{cells}{ml}$
Oxygen production	$200 \frac{fmol\ O_2}{cell \cdot h}$
Temperature	$30\ °C$
Pressure	$\sim 81\ kPa$

The cell total count in the volume of 1 liter would therefore be

$$n_{cells} = 10^6 \frac{cells}{ml} \cdot 1000ml = 10^9\ cells$$

This would equal a total rate of oxygen produced by the algae of

$$\dot{n}_{O_2} = 200 \cdot 10^{-15} \frac{mol\ O_2}{cell \cdot h} \cdot 10^9\ cells \cdot 24 \frac{h}{d} = 0.0048\ \frac{mol\ O_2}{d}$$

The standard amount of oxygen in Boulder at the beginning of the test is:

$$n_{O_2} = \frac{p \cdot V}{R \cdot T} = 0.21 \cdot \frac{0.8 \cdot 101325\ Pa \cdot 0.005\ m^3 \cdot mol \cdot K}{8.314472\ J \cdot 303.15\ K} = 0.0338\ mol\ O_2$$

This would equal a change of oxygen concentration over one day of

$$\frac{0.0048\ mol\ O_2}{0.0338\ mol\ O_2} = 3\ \%$$

Due to these findings it was concluded, that with a test duration of 1-2 days a measurable change in oxygen and carbon dioxide concentration can be achieved.

5.2. Support culture

Chlorella vulgaris was purchased from Carolina Biological Supply Company in bacteria free cultures. Immediately after receiving, the container was opened and the concentrated algal culture was diluted with 200 ml of Alga-Gro solution also procured from Carolina Biological Supply Company. The batch was put in graduating cylinders and provided with light in 12/12 cycles as seen in Figure 8. The entire set up was covered with a cardboard box that inhibited room light from disturbing the 12/12 cycle but allowed gas to exchange between the solution and the environment.

Figure 8: Batch culture set up

Since the algal solution was exposed to the environment and the lamp generated heat, there was a loss of water due to evaporation of about 2 ml/d. Approximately every second day a sample of about 10 µl was taken. With that a cell count was performed using a hemocytometer. The chlorella vulgaris cells in the red squares shown in Figure 9 were then counted in a microscope at a magnification factor of 40. The average was then multiplied with a factor of $1.6 \cdot 10^5$ to get the density in cells/ml.

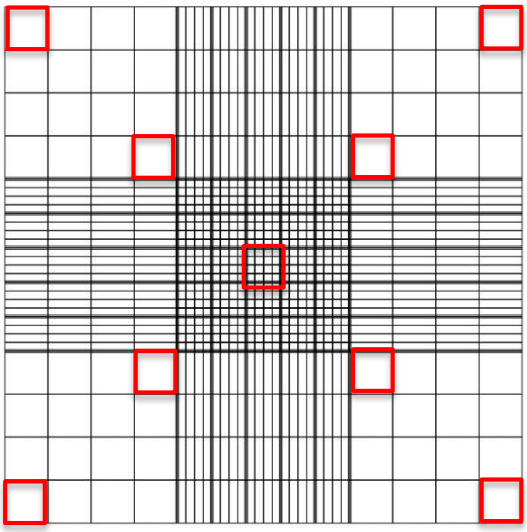

Figure 9: Schematic of hemocytometer with measurement areas shown in red

Due to different batch volumes and the separation of the algal cells in different cylinders also the total amount of cells were measured and are represented in Figure 10. It can be seen that the hemocytometer is a very reliable tool, as on day 8 and 12 the measurements immediately before and after dilution are really close to each other. The drops in the population density are also caused by the dilution. As seen in the graph the cell count was rising for the first 10 days. Dilution occurred for the first time on day 8. The population density kept rising immediately after that but suddenly decreased on day 12 right before the second dilution. From there the cell count dropped back to initial levels. Even though the reason for this behavior is unclear different theories exist. First an offset between the dilution schedule and the time of maximum population density could have led to inhibited cell doublings. The highest population density achieved was reported to be 10^9 cells/ml (Lee and Palsson, 1994). The second theory is an error induced through sampling procedures. An increase in cell count is followed every third data point. Mixing through sampling could potentially have influenced the growth behavior of the culture.

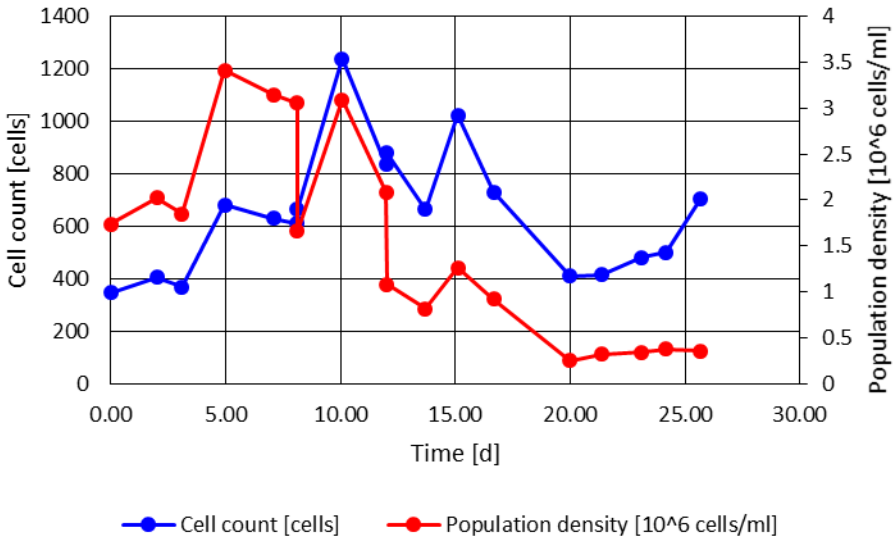

Figure 10: Cell count and population density of the batch culture

To assure that there would be sufficient algal cells for each run a culture system was developed and is partly shown as a Gantt chart in Figure 11. The plan was to conduct a total of nine tests. According to the pilot study each of the three cases (none, stirred and sparged) were planned to conduct three times to show repeatability. Developing a test schedule while dealing with algae is crucial. Only if there is consistency between the initial population densities of the individual tests can they be used for comparisons. Additionally, the algae have to be taken out of the batch culture from the same phase. Directly after arrival the cells need about three days for adaptation before they start doubling. After ten days the culture can be divided and diluted to establish two separate culture lines. This allows testing twice a week but also provides redundancy in case a batch goes bad before testing is finished. After a total of two weeks a critical volume is reached to support both a 1 liter experiment as well as a 1 liter subculture. The second culture line has an interim culture to establish a time offset between the two lines. The schedule also addresses the fact that algae cells should always be sampled from the log phase. This also is important to establish a fast adaptation to the elevated carbon dioxide during the test and establishes a baseline

between tests. The original test plan was to test twice a week for 48 hours. As a result of an unexpected leak (see section 5.3) during the first test cycle the test plan had to be redesigned. Beginning from test 4 there were no more algae involved in the testing and the schedule became flexible.

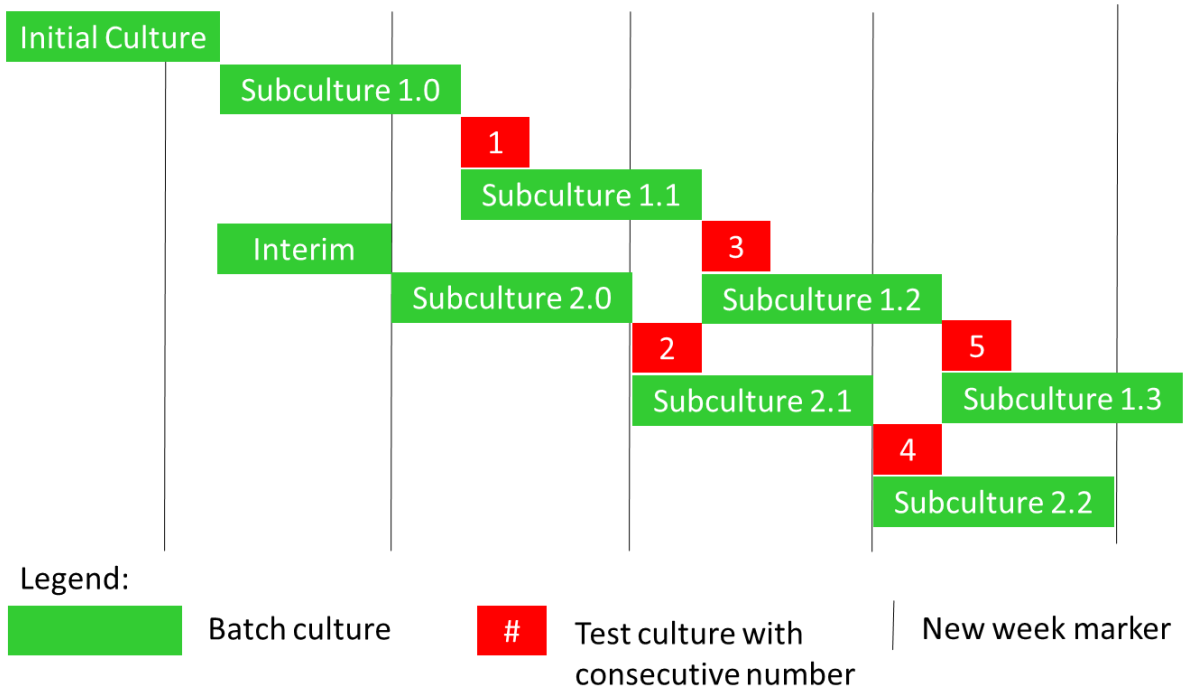

Figure 11: Gantt chart of planned culture and test timeline

5.3. Recirculated system

In order to conduct the experiment a test set up was developed. As mentioned in chapter 2 the set up was designed as a closed loop instead of an open loop system. A system schematic can be seen in Figure 12. The appropriate air composition is provided by AETHER only for the initial 5 minutes to purge the entire system with shop air enriched to 6 % carbon dioxide. A manual valve coupled with a shutoff of the AETHER air stream then gets closed and completely seals up the chamber loop. The chamber is filled with 1 liter of algal solution. From there an air pump pulls air

from the headspace of the chamber and pushes it into the outlet part of AETHER were the analysis of the gas composition is performed. In the usual concept of operations this gas is then vented but in this experiment it is fed back into the chamber. Inside the beaker a diffusor tube on the floor of the chamber than distributes the gas stream in small bubbles to gain a higher surface area for gas exchange but also thorough mixing of the algae. To complete the set up, the chamber was placed into a water bath that was heated to 30 °C. Due to a really small heater and a pyro foam insulation around the entire water bath there were no measurable swings in the temperature. The used light had an intensity of 810 lumens at a spectrum of 6000 K (cool white) and was activated in a 12/12 light/dark cycle. It is important to note that this test was performed at Boulder with an atmospheric pressure of about 85 kPa. This is also the pressure within the chamber as it is purged under ambient conditions at the beginning of each test. Finally a magnetic stir plate was added below the insulation with the actual stirrer within the chamber to allow mixing. A summary of all constant variables can be found in Table 6.

Table 6: Constant variables of the test set up

Variable	Value
Temperature	$30\ °C$
Initial Carbon Dioxide Concentration	$6\ \%$
Light intensity	$810\ lumens\ (\sim 3000\ lx)$
Light cycle	$12/12\ hours$
Light spectrum	$6000K\ cool\ white$
Initial algae density	$5 \cdot 10^5 \frac{cells}{ml}$
Volume of algae solution	$1\ l$

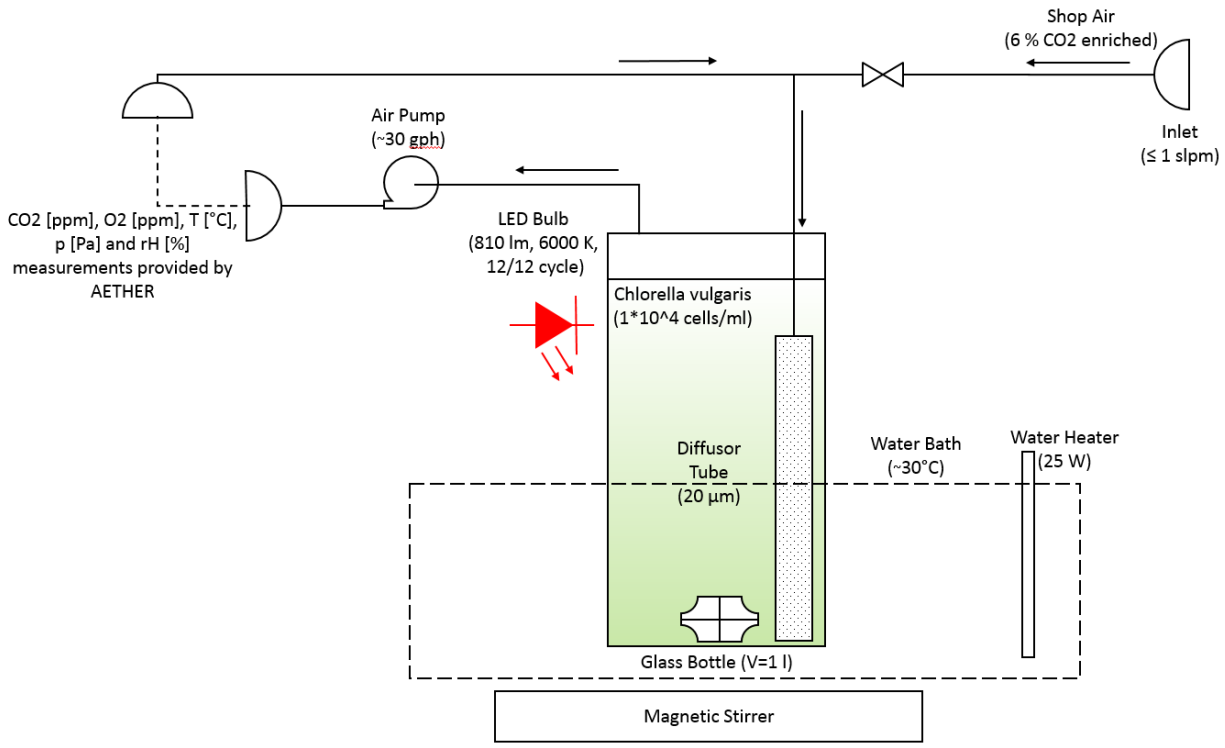

Figure 12: Recirculated flow test set up

In Figure 14 one can see an overview of the carbon dioxide levels versus time for the different tests performed. In all the tests it was tried to achieve a concentration of 6 % carbon dioxide. Firstly a test without algae was started as reference for potential leakage which was determined to be a gas exchange of the entire volume in four days. After that the test was conducted with algae once sparged and once stirred as seen in Figure 13.

Figure 13: Recirculated flow test with algae in the sparged set up

In the sparged case (test 2) the carbon dioxide decay was comparable to the reference case (test 1) but in the stirred case (test 3) there was a sudden drop in carbon dioxide. In order to identify the leak source different carbon dioxide decay tests within the loop were performed without algae. In order to find the source for the leakage one component was detached after another. Test 4 tested the set up without AETHER connected to the loop. Solely the flow-through carbon dioxide sensor was substituted for that. However the decay rate was comparable to test 3. Test 5 disconnected the chamber and just had the carbon dioxide sensor equipped with about 1 inch of capped Tygon tubing attached to each the inlet and outlet port of the sensor. Again the decay did not show any sign of change. This behavior was also seen in a repetition of this set up in test 6. It was concluded that therefore either the tube or the carbon dioxide sensor itself are the cause of the leak. Hence the seal of the carbon dioxide sensor was improved with J-B Weld and tested in test 7. Again it was the flow-through sensor with short plastic tubing. Unfortunately there was a mishap in the initial carbon dioxide concentration and the test started at only 1 kPa but also showed an initial drop. An important finding was the permeability of carbon dioxide through tubing. As seen in Cole-Parmer's tubing selection guide there is no available tubing that is a barrier for carbon dioxide (Cole-Parmer, 2015). However due to the design of other equipment which provide barbed fitting

interfaces it is required to use flex tubing. Tests 8 and 9 were conducted with the lowest carbon dioxide permeable tube which is FEP-lined Polyethylene. The graphs in Figure 14 however did not decrease their slopes compared to tests 5 and 6. Hence the low permeability tubing did not improve the leak rate. It is important to note that even though all these efforts were conducted the leak rate of 25 % from test 1 and 2 that compares to a carbon dioxide reduction rate of 0.05 %/h was never achieved again.

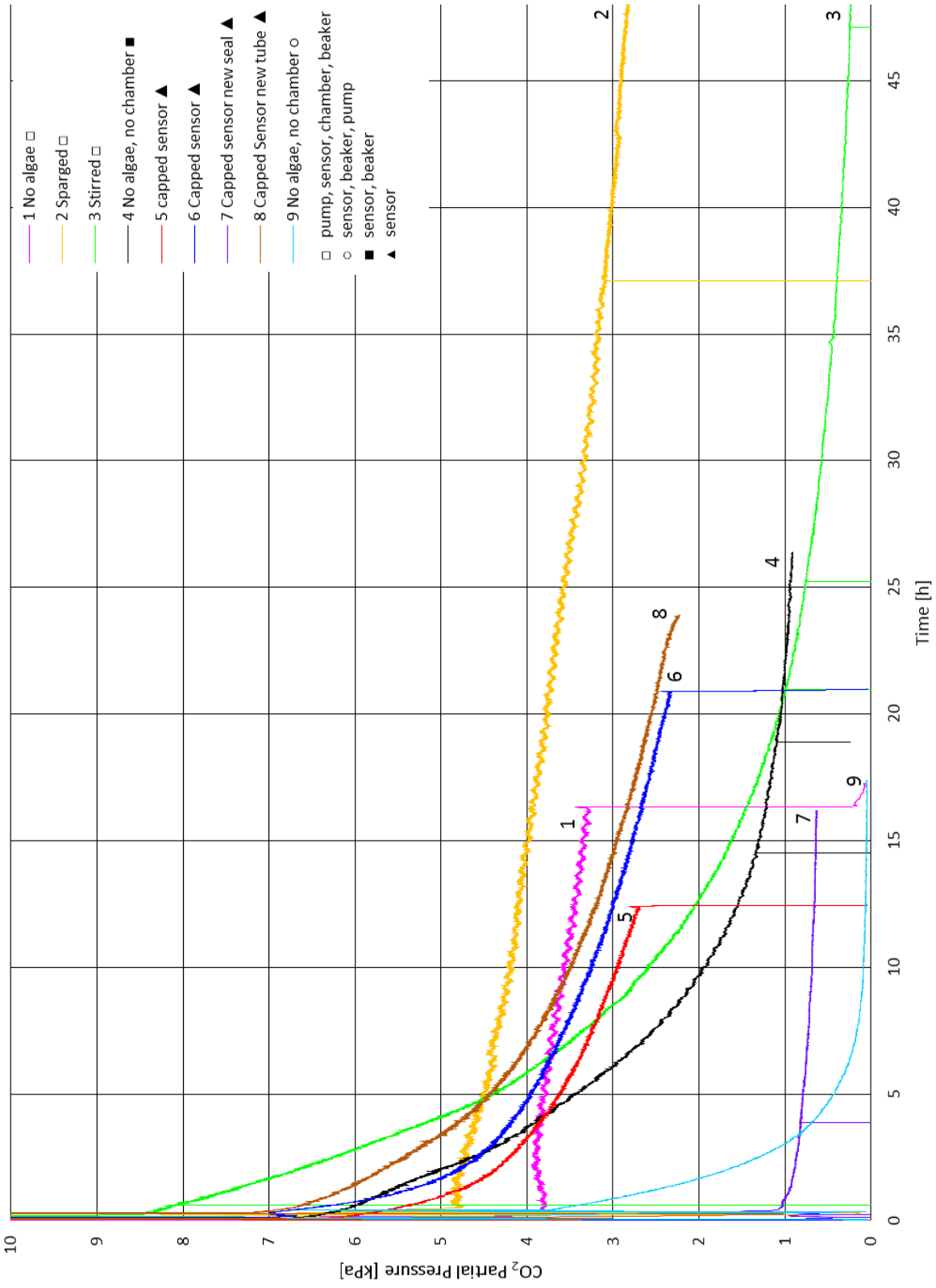

Figure 14: Partial pressure of carbon dioxide over time at the different decay tests

5.4. Closed container test set up

As seen in the recirculated flow set up all attempts to seal this system failed. At this point it was decided to change the set up. The new idea was to encapsulate the entire loop within a sealed chamber. For this a spray paint canister was found to be the correct quality and size. Additionally, it has a lid with a round gasket that provides a reliant seal to the environment. The algal solution, carbon dioxide sensor, pump and LED can fit inside the pressurized pot (Figure 15). The air pump is necessary to create the flow through the algal solution.

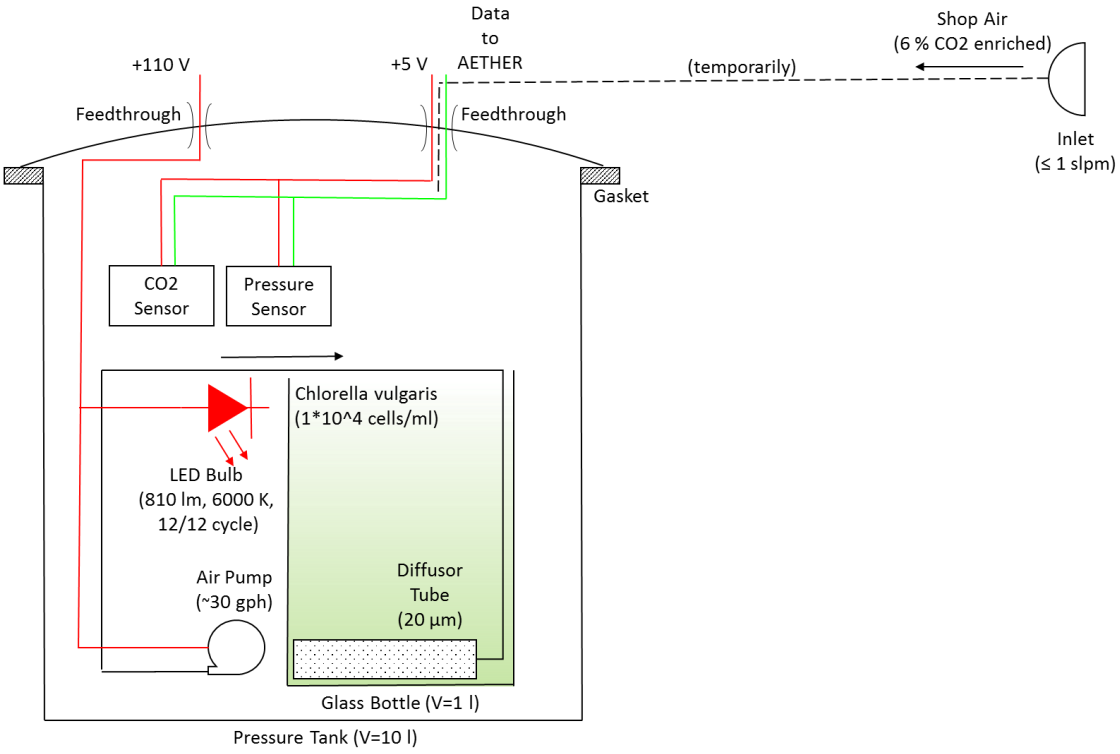

Figure 15: Closed container test set up

The initial purge of the entire pot with carbon dioxide can be performed with the lid partly installed, with the advantage that no separate port is needed for that. This eliminates a potential leak path. However power and data have to cross the container to interact with both the algae and

the AETHER DAQ system. For this special feedthroughs were designed. These feedthroughs are NPT thread plugs and a schematic can be seen in Figure 16. After inserting a hole along the axis the wires where placed into that. As there is a potential leak path between the copper wire and the FEP insulation, the wires were stripped a distance of about 5 mm. After assuring that there is no electrical continuity between the two wires the entire hole was filled with epoxy DP-100 and cured. Lastly the entire feedthrough was mounted into the lid, where the threads were sealed with X-PANDO pipe sealer.

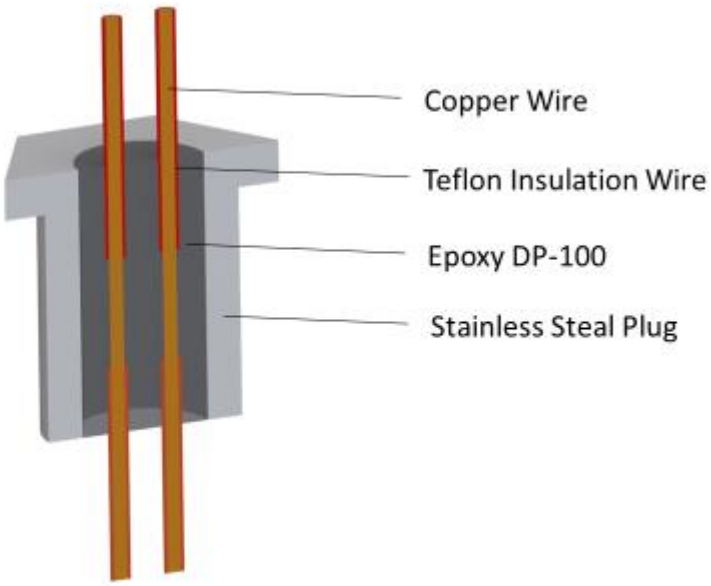

Figure 16: Schematic view of feedthrough

In order to test the leakage of this system the container was fed with carbon dioxide to about 3 kPa. The set up also included the air pump within the container to help mix the flow. The decay was then measured over a period of 66 hours as seen in Figure 17. This test shows a hermetically sealed system as there is no measurable decay of carbon dioxide over the entire test. The feedthrough design therefore was proven to be air tight. After this the test was repeated with

a beaker freshly filled with tap water as well as the LED light added. The partial pressure of carbon dioxide can again be seen in Figure 17. There is a big rise and drop of carbon dioxide with a factor of about 3 within the first 12 hours. The concentration than settled around 3 kPa for the next 12 hours before it again showed a drop and rise but this time in reversed order for another 12 hours. At the end it appears to be settling around 3 kPa again. Due to this very distinct behavior every 12 hours and the light cycle being on also in a 12/12 hours cycle the operating state of the LED was overlaid on the graph. It can be clearly seen that the light, that also causes an increase in temperature, is in correlation with the steady state parts of the graph. However at the start of the test the light was turned off and only water could have had an influence on the carbon dioxide level. As already known, water itself contains carbon dioxide in solution. One possibility of the initial raise of carbon dioxide is the heating up of cold tap water to room temperature. With higher temperatures the solubility of carbon dioxide in water decreases and carbon dioxide is released. To verify this assumption the following calculation was performed:

$$m_{CO_2} = \frac{p \cdot V}{R_s \cdot T} = \frac{3000\ Pa \cdot 0.0085\ m^3}{296.839\ \frac{J}{kg \cdot K} \cdot 294\ K} = 0.3g$$

The solubility of water is 1.6 g carbon dioxide at 21 °C whereas at tap water with a temperature of 15 °C is 2.1 g (Toolbox, n.d.). The difference of 0.5 g carbon dioxide is therefore released into the container to 0.8 g total. Converting that back into a partial pressure is shown below.

$$p_{CO_2} = \frac{m \cdot R_s \cdot T}{V} = \frac{0.008\ kg \cdot 296.839\ J \cdot 294\ K}{0.0085\ m^3} = 8214\ Pa$$

This correlates with the observed spike of carbon dioxide to about 9 kPa seen in the graph. Potentially a cool down of the lab to about 15 °C could have occurred in the middle of the night explaining the decrease of carbon dioxide between hour 6 and 9 due to absorption. However at latest when the light comes on the temperature raised to more than 21 °C but carbon dioxide remained constant at 3 kPa which brakes that theory.

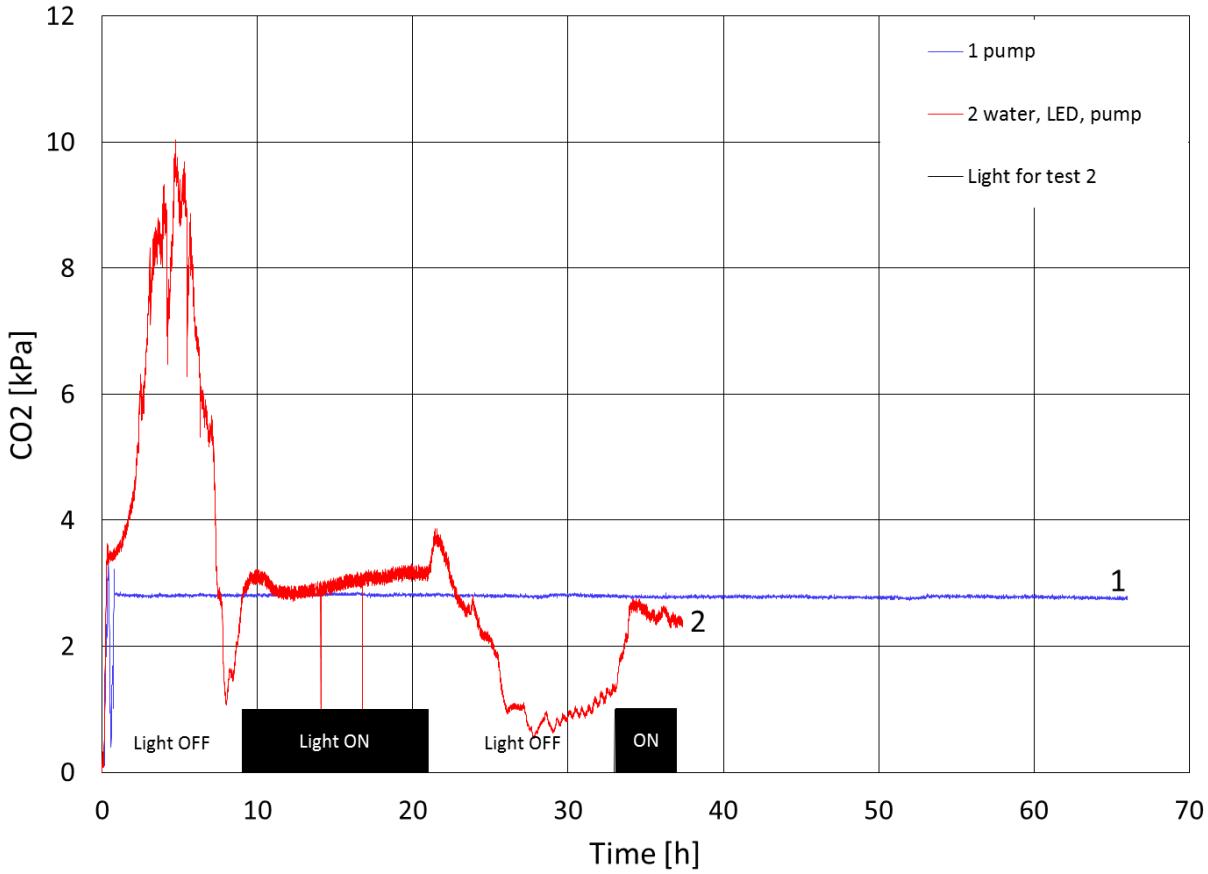

Figure 17: Partial pressure of carbon dioxide over time in the pressure pot set up

6. Discussion

6.1. Summary

In this thesis the goal was the evaluation of a flow-through test set up for experimental investigations on algal flow-through applications. Due to literature research the highest photosynthetic efficiency was found to be at a carbon dioxide concentration of 6 %. However, most ground based systems as well as the proposed water walls concept all plan to continuously operate under ambient carbon dioxide conditions (Belz et al., 2013; Chaumont, 1993). As minimal mass of water is critical for the success of this technology it seems reasonable to suppose that the reactor should be operated at the highest conversion rate. As this requires a carbon dioxide concentration of 6 % a photobioreactor might also be utilized as a secondary process comparable to the Sabatier reactor nowadays. This also has effects on the design of the photobioreactor as the former function favors a continuous operation whereas the latter favors a cycled operation.

During the early stage of the research project it was clear that the expected changes in gas compositions were challengingly small to find accurate measurement devices. Algal resources for increasing the algal photobioreactor to increase the expected changes also were limited. In order to conduct the test, the set up was changed to a recirculated flow. This allows to detect the differences in gas composition due to extending the reaction time but also gives the opportunity to easily convert it into a flow-through system. A series of tests were started to perform but the carbon dioxide decay in the system due to leakage was higher than expected. With further tests trying to isolate the problem the leakage got even worse. One of the major problems associated with that was the design of the air pump and carbon dioxide sensor that required flexible tubing. However it was found out that all available flex tubing is permeable to carbon dioxide to some extent. In order to resolve this problem the entire loop was placed in a sealed container. After the seal of the

container was verified by a carbon dioxide decay test, correlations between temperature as well as carbon dioxide solubility in water and carbon dioxide levels were observed.

By trying to evaluate the feasibility of utilizing flow-through testing for measuring carbon dioxide reduction and oxygen production rates of algae it has to be concluded that the feasibility is not proven yet. This statement is founded by the fact that it was not yet possible to develop a stable system that could demonstrate functionality. However, with improvements laid out in section 6.3 it is likely to develop a functional system.

6.2. Lessons learned

During the testing a lot of issues were found that required further investigations. Below is a list of the most important findings.

6.2.1. Solubility of carbon dioxide in water

When the algal solution is not handled under steady state conditions the absorption and desorption of carbon dioxide in water mainly dictates the carbon dioxide concentration in the chamber. Especially with varying temperature, pressure or both the carbon dioxide reduction from algae and absorption of carbon dioxide from the water can happen separated from each other. Over the long term of course the photosynthetic activity of algae is of course dependent on the concentration of carbon dioxide in the solution.

6.2.2. Permeability of flexible tubing

An issue that was not recognized at the beginning of the experiment was the permeability of flexible tubing to carbon dioxide. Mainly with the selection of the tubing one can already change the permeability by three orders of magnitude as described in the literature (Cole-Parmer, 2015). However it is important to note that there was not a single tube found that is not permeable to carbon dioxide.

6.2.3. Low resolution of differential measurements

The differences in oxygen and carbon dioxide concentration in a small scale flow-through set up are much smaller than in comparable physicochemical systems. There is the possibility of either increasing the volume of the algal solution or preferably increasing the density of the algal solution. Also it becomes quite challenging to find a sensor that can measure around 6 kPa carbon dioxide but is accurate to less than 0.01 kPa at the same time.

6.2.4. Evaporation of algal solution

The evaporation in the batch culture that was used to cultivate algae was higher than expected. This was especially a problem as the population density was used to compare algal growth, which changes depending on the total volume of the algal solution. In a flow-through system evaporation is occurring at an even higher rate and will be a concern.

6.2.5. Adhesiveness of algal cells to surfaces

The hemocytometer has proven to be a very good method to determine the population density in the batch culture. By stirring the solution the algae loosen from the surface and go into a uniform solution. However it was shown that due to the algae preference for surfaces it is more difficult to follow that approach in the set up with the diffusion tube. It might be possible to loosen the algal cells from the outside but there is no way of getting the algal cells that might grow inside the diffusion tube back in solution. The test set-up was not designed for taking measurements and drying out the algal solution. This would have allowed the alternative approach of weighing the produced biomass.

6.3. Design recommendations

All the errors encountered, experienced and investigated led to design recommendations for a future flow-through set up. This kind of set up is highly encouraged as it allows measurements under steady state conditions. Compared to a recirculated flow that has a decay processes associated with, it creates the potential to control the confounding variables to constant variables. This is beneficial in the current research step as experiments can be isolated to one independent variable. At a later point this can be increased to allow tests of integrated systems.

6.3.1. Utilization of metal tubing

Firstly, due to the lack of non-permeable flexible tubing, stainless steel tubing compression fitting should be used as they are non-permeable to carbon dioxide.

6.3.2. Provision of lower flow rates

Secondly AETHER should be adapted and verified to also provide gas compositions at lower than 1 slpm to increase the differences in carbon dioxide concentrations that can be measured across inlet and outlet. A suitable flow rate would be less or equal than 100 ml/min.

6.3.3. Higher resolution gas analyzer

Current sensor equipment provided by AETHER also is not precise enough to identify changes so small. To allow measurability of the gas composition it is crucial to upgrade AETHER with new gas analyzers that provide a resolution of about 100 ppm. Scientific multi gas analyzers as for example provided by CEA Instruments, Columbus Instruments or California Analytics are required. Even though increasing the volume of the algal chamber would increase measurability it is not recommended to increase the size to more than 4 l. In large volumes shadowing becomes a more important factor. Also differences within the chamber are increasing due to settling of cells. Lastly most investigated papers also fell in this range and allow comparability.

6.3.4. Temperature tolerant photobioreactor

Even though the experiments can be conducted with solely the gas composition data a very applicable measurement would be the biomass. This is not just a verification for the compositional measurements but also adds value to the work by adding comparability with previous work. As algae are difficult to separate from the bioreactor, designing the entire reactor in a way that it can be heated up to 80 °C to evaporate the water and measure the biomass is required.

6.3.5. Consistent solution volume

In order to prevent inadvertent evaporation during the flow-through experiments either a vapor seal or a water trap has to be installed in the set up.

6.3.6. Increase of contact time between gas flow and algal solution

Another attempt to improve this system could be through increasing the contact time between the air stream and the algal solution. Especially with the goal of developing this system for a microgravitational environment it would allow an increase of carbon dioxide reduction in the air stream. With a maze pattern or a design similar to a shell and tube heat exchanger the surface area as well as carbon air stream dispersion can be increased.

6.3.7. Introducing strobe light effect

As indicated in the literature a strobe light effect at a frequency of 100 Hz can be added to increase efficiency (Liao et al., 2014).

6.3.8. Utilizing carbon dioxide buffer provided by water

Lastly, observations of the adsorption and desorption of carbon dioxide in water could be used as a design feature in a potential bioreactor. By cooling down the algal solution, high carbon dioxide loads can be buffered during high activity periods as well as during emergency situations.

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Appendix

A. Timeline

08/25/2014 Literature review started

10/07/2014 Culturing set up for two 4 liter samples finished. Set up included darkened box, glass beaker and a 810 lm LED lamp that was programmed to a 12/12 ON/OFF cycle.

10/08/2014 Two chlorella vulgaris samples (10 ml each) received from Carolina and mixed in 200ml of distilled water and 4 ml of Alga-Gro solution. One cylinder was mixed through with a spatula every 3-4 days, whereas the other one was remained still.

10/24/2014 First 100 µl sample taken from batch to determine cell count with hemocytometer (2.8624•10^6 cells/ml).

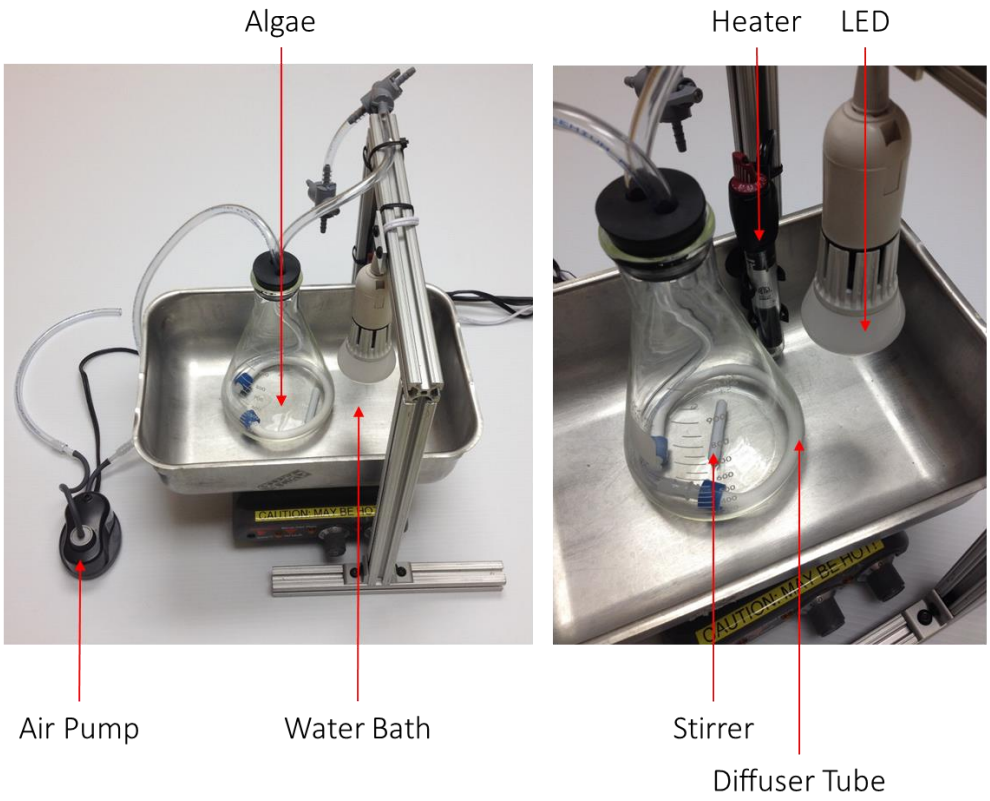
11/10/2014 Batch was moved from Bioastronautics lab to BioServe cell culture lab to achieve a more consistent environment due to a smaller lab volume and less frequent use.

11/17/2014 Start seeing clumping of unicellular algae cells under microscope.

12/01/2014 Algae lost color and are dead.

01/13/2015 Chlorella vulgaris (10 ml) received from Carolina and mixed in 200 ml of distilled water and 4ml of Alga-Gro solution. Same test set up was used as in initial batch, but solution was stirred every 1-2 days. Initial cell density was 2.027•10^6 cells/ml

01/23/2015 Algal closed loop test set up finished. Including a temperature controlling water bath, peristaltic pump to recirculate air into algal chamber and sensor and magnetic stirrer.



01/26/2015 Presentation given to LifeLAB team

01/30/2015 First test on AETHER rig (algae sparged in beaker, connected to sensor chamber with pump running, 48 h)

01/29/2015 Set up tested with inserting carbon dioxide (6 %) and measuring decay (16 h)

02/08/2015 Second test on AETHER rig (algae stirred in beaker, connected to sensor chamber with pump running, 48 h)

02/12/2015 Third test on AETHER rig (no algae, beaker connected to sensor, no sensor chamber, no pump, 27 h)

02/16/2015 Fourth test on AETHER rig (no algae, no beaker, no sensor chamber, no pump, just capped of sensor, 14 h)

02/24/2015 Fifth test on AETHER rig (no algae, no beaker, no sensor chamber, no pump, just capped of sensor, with longer wait time between stopping flush air and capping line, 21 h)

02/27/2015 Sixth test on AETHER rig (no algae, no beaker, no sensor chamber, no pump, just capped off sensor especially sealed around ports, 16 h)

03/05/2015 Seventh test on AETHER rig (no algae, no beaker, so sensor chamber, no pump, just capped off sensor but with new low permeability tube, 24 h)

03/10/2015 Eighth test on AETHER rig (no algae, no sensor chamber, pump connected with sensor and beaker, 17 h)

03/20/2015 New pressure pot set up finished.

03/20/2015 Leak test of new pressure pot set up.

03/27/2015 Repeat of leak test of new pressure pot set up.

04/17/2015 Oral defense

04/23/2015 Master's thesis submission