Bachelor-Thesis an der Hochschule Luzern - Technik & Architektur

Titel	**Construction of an algalbioreactor for space habitats**
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Bachelor-Studiengang	**Bachelor Maschinentechnik**
Semester	**FS19**
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Abstract Deutsch
In einem kleinmassstäbigen, geschlossenen System wie zum Beispiel in einem Habitat im Weltraum, ist die Nachhaltigkeit innerhalb des Systems besonders wichtig. Einige Mikroalgen-Arten verfügen über die Fähigkeit Licht und CO 2 innerhalb des Habitates nutzbar zu machen, um photosynthetisch Sauerstoff zu generieren. Hier werden das Konzept sowie auch die Konstruktion eines Flachplatten-Bioreaktor (mit einem Volumen von min. 10 Liter) dokumentiert. Diesen Prototyp kann man für die Forschung im Labormassstab einsetzen. Die Hauptkomponenten sind der Kultivierungsbehälter und die externe Sensorenkammer. Das Algenmedium zirkuliert zwischen diesen zwei Hauptkomponenten mit Hilfe einer Membranpumpe. Die Sensorenkammer besteht aus einem 3d-gedruckten Gehäuse aus PLA und einem Sensoren-Array, welches die pH-Werte, die Konzentration von gelöstem Sauerstoff im Medium, Temperatur und die optische Dichte (Fluoreszenz/Chlorophyll-Zellen) misst. Das Gesamtsystem besitzt eine modulare Bauweise und ist teilweise sterilisierbar. Mit diesem Prototyp ist es möglich, neue Methoden zu entwickeln, die die Wachstumsrate und Biomassenkonzentration von Mikroalgenzellen verbessern, um einen Carbon-Negativen Prozess zu erreichen.

Abstract Englisch
On small-scale, closed system such as a habitat in outer space, the complete sustainability inside the system is highly sought after. Some microalgae have the ability to harness the light and use the CO 2 from inside the habitat to photo-synthetically produce oxygen and take up liquid human waste. This work documents the details of the construction of a ten-liter flat-plate bioreactor system that has been designed for experiments at the laboratory scale. The system consists of one cultivating vessel, a dedicated sensor chamber. The algal medium is continuously pumped out of the vessel to the sensor chamber, before routed back into the main tank via a diaphragm pump. The sensor chamber is fitted with probes that measures pH, dissolved oxygen, temperature (in main tank and the sensor chamber), the optical density of the medium and the chlorophyll fluorescence. The reactor is modular and sterilizable. Based on the current reactor design, the operation of the system should enhance growth rate and biomass concentration of microalgae cells with low power inputs creating a carbon negative process.

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Construction of an algal bioreactor for space habitats

Bachelor Thesis

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Zusammenfassung

In einem kleinmassstäbigen, geschlossenen System wie zum Beispiel in einem Habitat im Weltraum, ist die Nachhaltigkeit innerhalb des Systems besonders wichtig. Einige Mikroalgen-Arten verfügen über die Fähigkeit Licht und CO_2 innerhalb des Habitates nutzbar zu machen, um photosynthetisch Sauerstoff zu generieren. Hier werden das Konzept sowie auch die Konstruktion eines Flachplatten-Bioreaktor (mit einem Volumen von min. 10 Liter) dokumentiert. Diesen Prototyp kann man für die Forschung im Labormassstab einsetzen. Die Hauptkomponenten sind der Kultivierungsbehälter und die externe Sensorenkammer. Das Algenmedium zirkuliert zwischen diesen zwei Hauptkomponenten mit Hilfe einer Membranpumpe. Die Sensorenkammer besteht aus einem 3d-gedruckten Gehäuse aus PLA und einem Sensoren-Array welches die pH-Werte, die Konzentration von gelöstem Sauerstoff im Medium, Temperatur und die optische Dichte (Fluoreszenz/Chlorophyll-Zellen) misst. Das Gesamtsystem besitzt eine modulare Bauweise und ist teilweise sterilisierbar. Mit diesem Prototyp ist es möglich, neue Methoden zu entwickeln, die die Wachstumsrate und Biomassenkonzentration von Mikroalgenzellen verbessern um einen Carbon-Negativen Prozess zu erreichen.

Abstract

On small-scale, closed system such as a habitat in outer space, the complete sustainability inside the system is highly sought after. Some microalgae have the ability to harness the light and use the CO_2 from inside the habitat to photo-synthetically produce oxygen and take up liquid human waste. This work documents the details of the construction of a ten-liter flat-plate bioreactor system that has been designed for experiments at the laboratory scale. The system consists of one cultivating vessel, a dedicated sensor chamber. The algal medium is continuously pumped out of the vessel to the sensor chamber, before routed back into the main tank via a diaphragm pump. The sensor chamber is fitted with probes that measures pH, dissolved oxygen, temperature (in main tank and the sensor chamber), the optical density of the medium and the chlorophyll fluorescence. The reactor is modular and sterilizable . Based on the current reactor design, the operation of the system should enhance growth rate and biomass concentration of microalgae cells with low power inputs creating a carbon negative process.

List of commonly used Abbreviations and Symbols

Acronyms and Abbreviations

ABS	Acrylonitrile Butadiene Styrene
BR	Batch reactor
CFD	computational fluid dynamics
CSTR	Continuous stirred tank reactor
DO	Dissolved oxygen
DOT	Dissolved oxygen tension
Eawag	Swiss Federal Institute of Aquatic Science and Technology
ESA	European Space Agency
FSC-H	Forward scatter-height
GSM	Global System for Mobile communications
GUI	Graphical user interface
LED	Light-emitting diode
N	Nitrogen atom
NBR	Nitrile rubber
NC	normally closed
NO	normally open
OD	optical density
P	Phosphorus atom
PFR	Plug flow reactor
PIV	particle image velocimetry
PLA	Polylactic Acid
PSU	Power supply unit
PWM	Pulse Width Modulation
S	Sulfur

SSC	Swiss Space Center
SSC-A	Side scatter-area
UBEC	universal battery eliminator circuit
VAC	Volts of alternating current
VDC	Volts of direct current

Symbols

ρ	Density of medium (is assumed to be equal to water density: 1000 kg/m^3)
g	Gravitation acceleration (= 9.81 m/s^2)
A	Area [m^2]
$\varnothing$	Inside diameter (unless indicated otherwise)

Contents

Chapter 1

Introduction

1.1 Motivation

One of the most important essentials for human in space travel and planet colonization is the breathable atmosphere, together with water, food, and even the sensation of weight. Engineering criteria to assure physiological safety and comfort are essential, but equally important is to provide for psychological and esthetic needs of the colonists [2].

To maintain life processes adequately, the astronauts requires an atmosphere of acceptable composition and pressure. The atmosphere of the habitat must not have high levels of carbon dioxide ($CO_2 > 1\%$), so this gas must be scrubbed to lower its concentrations. The partial pressure of oxygen (pO_2) must be sufficient to provide high enough partial pressure within the alveoli of the lungs (~13.4 kPa or ~100 mmHg) for good respiration. Yet this must be low enough to avert losses in blood cell mass and larger changes in the number and distribution of micro-organisms, such as the growth of "opportunity" bacteria [3]. As a part of the demonstration project **IGLUNA** of *ESA_Lab* from European Space Agency (ESA) and Swiss Space Center (SSC), a supplemental oxygen generator and CO_2 uptake process will use an algal bioreactor integrated into the demo habitat in Zermatt, Wallis, Switzerland. This bioreactor will act as a fail-safe life support/oxygen generation system for the habitat.

Inside the habitat, bio-waste such as bodily wastes, and food scraps can be treated by pyrolysis or torrefaction process. The gas emissions from these processes consists mainly of flammable gases (such as CO, H_2, CH_4), non-flammable gasses (such as N_2, CO_2, H_2O), and gaseous impurities: NH_3, HCN, H_2S, COS (Carbonyl sulfide). By utilizing these products, the algal bioreactor could theoretically remain active for a significant long time span and passively produce oxygen via photosynthesis.

Lastly, algal biomass is rich in biomolecules that can be processed to industrial bioproducts such as bioplastics and carbon fibers. These types of products are important for additive manufacturing of new components on space stations, where logistics of shipping materials from Earth is restricted.

The algae, which is cultivated for this project, is *Emiliania huxleyi*. This microalgae is a coccolithophore, with approximately 30 calcite plates ($CaCO_3$) over its surface, and therefore it's a sink for carbon. The algae is completely autotrophic and sequesters carbon dioxide (CO_2) from the atmosphere to drive photosynthesis (i.e. more biomass) and calcification (more $CaCO_3$).

- It has an approximately spherical diameter of $7\mu m$ (FSC-H = $1.1x10^6$)
- The debris is smaller than 0.7μ (based on a forward light scatter, FSC-H = $9.5x10^4$)
- The delta between algae and the debris would be larger, if the algae were aggregation (clumping).
- The biomass can also be extracted for lipids for biofuel, carbohydrates for bio-plastics, pigments for medical and health treatments, and a variety of enzymes and polymers for industrial processes.

1.2 Scope of work

While this work's main purpose is to deliver a functional prototype to the project IGLUNA solely for conceptual demonstration, the prototype would however be used for further research on algae at the Competence Center for Bioscience and Medical Engineering in Hergiswil, Switzerland.

Initial situation

The original requirements of the project and their status (as of March 2019):

1. A concept design of a 10 liter bioreactor, which would be illuminated, using an existing custom-made LED arrays (with blue and red lights) shall be established. [done]
2. The concept shall consist of a system of baffles to improve the mixing process inside the reactor (by generating vortices). [planned]
3. A sensor array and program controller shalle be assembled to operate the bioreactor and measure temperature, chlorophyll fluorescence, O_2 , pH, optical density (OD) at rate of once per minute for the duration of the demonstration. [done]
4. A gas/nutrient infusion shall be conceptualized and reliazed to systematically and periodically saturate the algal medium. [open]

5. Test growth rates of algal strains in the lab under simulated conditions (constant temperature, under specific lighting conditions). [planned]

For the final phase of the project, additional requirement inputs were added, after troubleshooting the initial planning:

6. The bioreactor concept shall be realized, integrated into the system, and set up for manual operations (with lighting, and hydraulic circulation)
7. Choosing and adjust design to fit the aeration device.
8. Design and construct an electrical switchboard, which contains the control system and power supply.
9. Program the control software for automated operation.
10. Trial run the system in laboratory before delivery.

This acts as a checklist for tasks, to be done in this thesis (From March 2019 to June 2019).

Chapter 2

Reactor concept

2.1 Design specifications

My motivation for designing the algal bioreactor was to create a prototype for the project "IGLUNA", demonstrating the feasibility of implementing such system inside a Lunar habitat. For demonstration purposes, the design shall be installed and operated inside a dedicated green house, under carefully controlled conditions. To accomplish this goal, various parameters were selected to be controlled and/or measured, which drive and optimize the final reactor design. The complete process required developing equipment and ancillary subsystems from the ground up, to operate the bioreactor. The significant parameters are temperature, optical density, fluorescence, dissolved oxygen, pH (which reflects CO_2). These parameters will be measured every minute and logged.

As a subsystem of the life support system, the bioreactor should be able to generate oxygen efficiently, while using the least amount of energy. By using the captured CO_2 from air [2], and utilizing nitrogen and phosphate from human waste [3], the reactor will grow algae continuously over time. Additionally, the proposed system should be able to integrate seamlessly into habitat, sharing its interface with other systems, such as hydroponic farms. To conduct the experiments successfully, the ambient temperature where the reactor is operating, should be around 15°C to 28°C.

The reactor was to be installed and operated inside a glacier [4], and later will be used for small-scaled experiments in the laboratory. To achieve this, a bench-top, modular format was selected to be easily operated in a lab environment, and to allow easy cleaning, maintenance as well as adjust test parameters between experiments. Other constrains in consideration for the

[2] For experimenting purpose, CO_2 will be supplied directly from a pressurized CO_2 tank.
[3] Processed human urine was provided by EAWAG in the form of the product Aurin.
[4] The glacier cave is at Zermatt, Canton of Valais, Switzerland

system were the bio-compatibility of construction materials, the wasted heat output (which leads the risk of melting the permafrost) and the fluid shear stress on algae cells, should be minimized.

2.2 Bioreactor design principles

There are a large number of photobioreactor designs suitable for cultivating microalgae in large volumes. These designs can be grouped into seven main designs [4] listed below.

1. Shaking flasks
2. Stirred tank (STR)
3. Horizontal tubular
4. Tubular coiled
5. Vertical tubular (bubble column/airlift)
6. Immobilization
7. Flat-plate

These bioreactors can be operated in several ways, for example as batch reactors (BR), continuous stirred tank reactors (CSTR), and plug flow reactors (PFR). Continuous stirred tank reactors provide the best option for maintaining exponentially growing cells i.e. in steady state. The other two operations give unsteady processes. To select the ideal design, the following parameters [5] were chosen:

V_R $[L]$ The total working volume of the reactor includes liquid and gas phase; the volumes of the pure liquid are usually not given as would be necessary for mass balancing.

A_R $[m^2]$ The total surface area of the transparent part of the reactor determines the amount of light which could eventually enter the reactor; detailed analysis is necessary, to calculate how much light can really hit the surface. The surface area makes a serious contribution to reactor cost.

A_G $[m^2]$ The aperture (ground) area of the reactor measures the area from which light energy is collected.

P_R $[gL^{-1}d^{-1}]$ The volumetric productivity $P_R = dm_X/(V_R \cdot dt_C)$ measures the product formation per reactor volume and time span. Lab scale experiments are often given on this volumetric basis.

P_G $[gm^{-2}d^{-1}]$ The areal productivity $P_G = dm_X/(A_G \cdot dt_C)$ is the most important parameter to assess larger photo-bioreactor. It allows for balancing in terms of energy efficiency between incident light as the main energy source

and biomass or product formation on an areal basis and is the determining performance criterion. This is especially true for conversion of solar energy to chemical energy e.g. bio-diesel produced by algae.

PCE [%] The photo-conversion efficiency measures the fraction of the light source energy that is converted to chemical during the cultivating process.

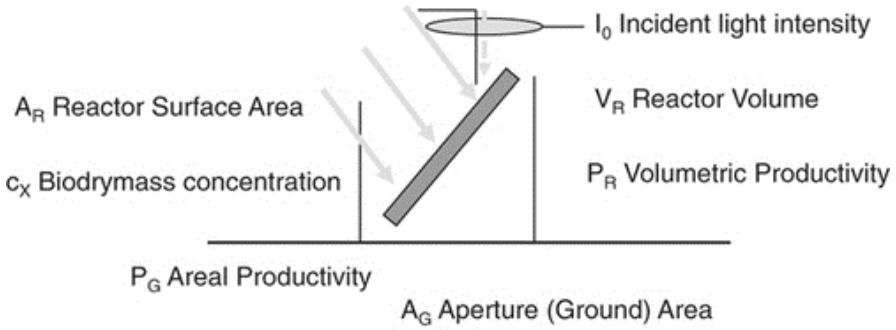


Figure 2.1: Bioreactor design parameters, definitions and items for assessment. [5]

Shaking flasks

Shaking flasks have been used in the study and optimization of biotechnology processes, allowing to the performance of experiments with minimal costs and material [6]. However, due to several limitations, such as the understanding of the involving factors (mixing/aeration), and the inability to monitor and control pH and/or dissolved oxygen tension (DOT), it's difficult to scale the system up to the test requirements for later large-scale production [7]. A practical scale-up strategy is cultivating the algae cultures inside a fermenter in batch and continuous mode. Another disadvantage of this design is, it's challenging for continuous gas collection, as the culture vessel is constantly under agitation by shaking.

For applications in space, the design is prone to failure due to higher number of moving parts.

Stirred tank (STR)

There are many different variations of stirred tank reactors: The simplest setup consists of bottles with magnet stir bars, carrying no sensors. The advantages of such design are they are very cost-effective and easy to handle, allowing conducting multiple experiments in parallel.

Its disadvantage is the low surface to volume ratio and due to mechanical agitation, it is energy-intensive. Moreover, it is inconvenient to scale up.

Horizontal tubular

The horizontal tubular type reactor is common for upscaled cultivation, due to high productivity [8]. The tubes giving a high surface to volume ratio and can be stacked in line to capture light optimally, although this require a larger land area.

Its disadvantages are the challenge of control the temperature and the low gas exchange and a significant gas gradient along the tubes caused by most of the gas exchange being conducted in a separate chamber. This design also requires high energy input and sometimes accumulates sedimented biomass in the tubes [5, 4, 8].

Tubular coiled

This design shares many similarities to the horizontal tube reactor type [9]. The advantages are high surface to volume ratio and with a conic helical shape, the light harvesting efficiency per land area can be increased.

Its disadvantages are high shear stress, accumulation of biomass in the tubes, low gas exchange, low operating volume, and a high energy input.

Vertical tubular (bubble column/airlift)

The main agitation method for this design is mixing by sparging of air. In lab-scale systems, heating-cooling coils or thermal jacket has been used to control the temperature. This design allows an efficient gas exchange, low shear stress, good mixing, and requires less energy and invest cost.

Its disadvantages are the inefficient light capture, and the low surface to volume ratio. This designs are inconvenient for oxygen production , since the agitation method depends on gas circulation (leading to higher risk of leakages) or gas addition (leading to additional gases separation).

Immobilization

Algae can be immobilized in matrices such as alginate beads, gel beads (see Figure 2.2), biofilm on solid surfaces, etc. This method has many advantages over the conventional liquid cultures: The films consist of a very thin layer of cells, results an excellent surface to volume ratio and a very short light path together with high cell density. There is no shear stress, and the cells are stored inside the gel, easy to be harvested.

The disadvantages are the high investment cost, and the difficulty to scale the system up.

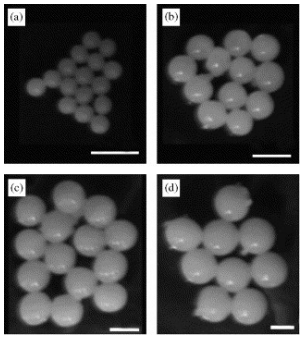


Figure 2.2: Photograph images of cell immobilized in barium alginate gel beads. The diameter of the beads: (a) 2 mm, (b) 4 mm, (c) 6 mm, and (d) 8 mm. The scale bars in all images represent 5 mm. [10]

Flat-plate reactor

Since the availability of light the limiting factor for algal growth, and since we want to determine the algal growth rate as well as its properties change under specific light wavelength, we selected the vertical flat-plate reactor as our main design. This format offers the highest surface-to-volume ratio. Moreover, compared with tubular-typed reactor , previous study [11] suggested that the flat-plate design was a better choice for oxygen production, due to factors such as a high efficient removal of the produced gas. Additional benefits of this design include flexible operating conditions, the feasibility of modular design and potential future scale-up for a space habitat.

Considering all required specifications, constrains, the flat-plate design was chosen. Further, a minimalistic design and the selection of ancillary systems, equipment and the construction material were made, to keeping the costs within the budget range. Using morphological box method, the selected reactor concept of the system is developed further and divided into many sub-functions, and then combined together to finalize the design. Main purpose of the work presented herein was to construct a photobioreactor to be used for oxygen production, carbon dioxide removal, and micro-algae cultivation.

2.3 Ancillary subsystems

These subsystems were concerned with illumination, agitation, measurement, flow control, data-logging, and a graphical user interface (GUI)(for monitoring and control).

- Illumination is provided by the green house module of the habitat [1]

[1]For this experiment, the prototype carries its own light-emitting-diode (LED) array

- Agitation method is sparging by air/CO_2 bubbles. To improve the absorption rate of CO_2, air baffles are installed inside the cultivating vessel (see Figure 2.3).

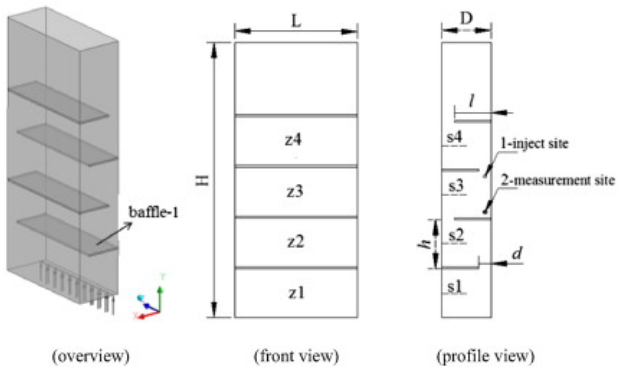


Figure 2.3: Schematic of the flat plate photobioreactor with horizontal baffles. [12]

- Measurement concepts:
  - Temperature, dissolve O_2, and pH: The proposed system uses commercial probes for *in situ* measurements.
  - Optical density (OD) and fluorescent/chlorophyll: Using narrow band LEDs in combination with photodiodes to monitor the algal growth rate (see Figure 2.4). The optical density of a medium describes the transmittance of light through that medium. This quantity is especially used in quantitative spectroscopy. The Beer-Lambert law describes OD as following, with I as light intensity:

$$OD = -log_{10}(\frac{I_{out}}{I_{in}})$$

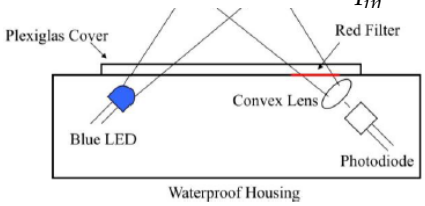


Figure 2.4: Concept of measuring the chlorophyll using narrow band LED

- Flow control: utilizing magnetic proportional valves to control the flow of the hydraulic system.
- Data-logging and GUI are implemented by means of a Raspberry Pi system.

Chapter 3

Construction

3.1 Overview

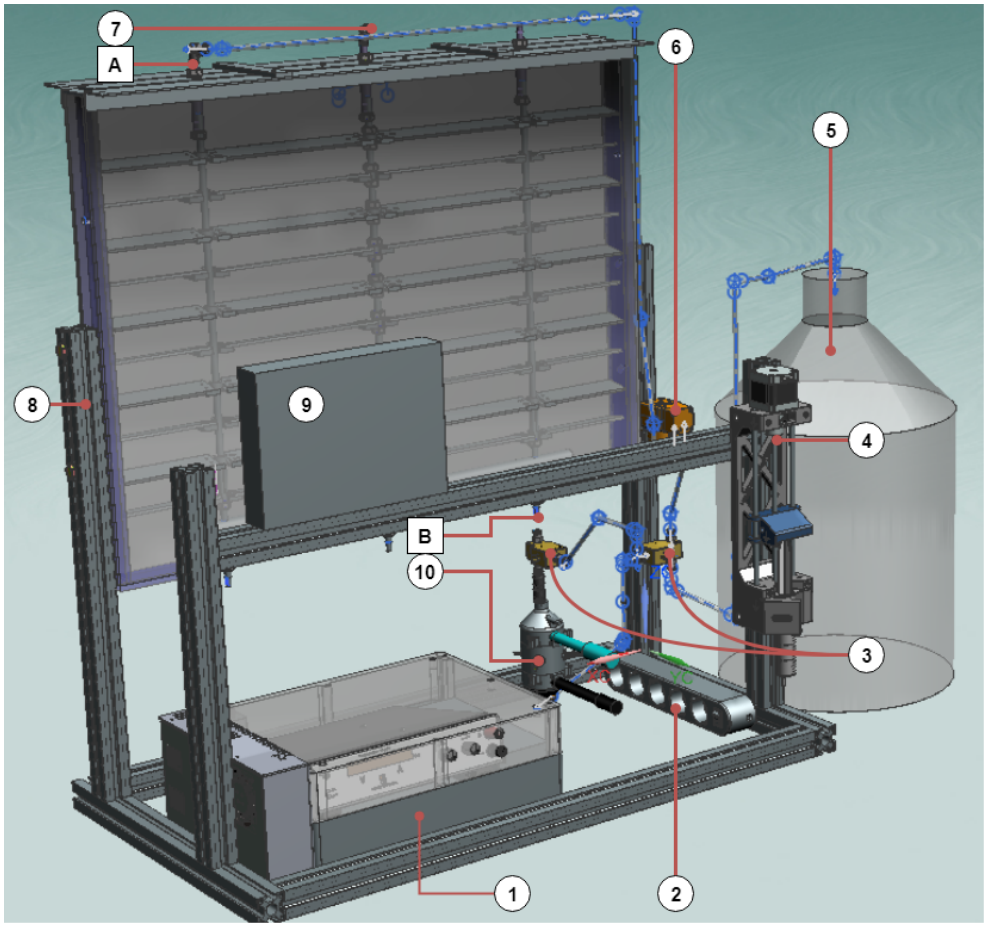


Figure 3.1: Bioreactor mock-up: (A) Algal medium inlet; (B) Algal medium outlet

1. Electronics housing: contains power supply units (PSUs), micro-controller and display.
2. Power strip with a rocker switch as emergency power off (EPO)
3. Magnetic, proportional 3/2 way: Bürkert-0127 Rocker solenoid valves (Type T) with separating diaphragm
4. Infusion spring pump (PatcherLab, CalTech)
5. Source tank: 15 Liters.
6. Diaphragm pump: KNF Flodos: NF 1.60 DCB
7. Gas inlet (CO_2 tank is not modeled).
8. Aluminum frame (Kanya AG)
9. LED arrays: custom-made for lab.
10. Sensor chambers: pH, temperature, O_2 and optical density (chlorophyll/fluorescent) sensors.

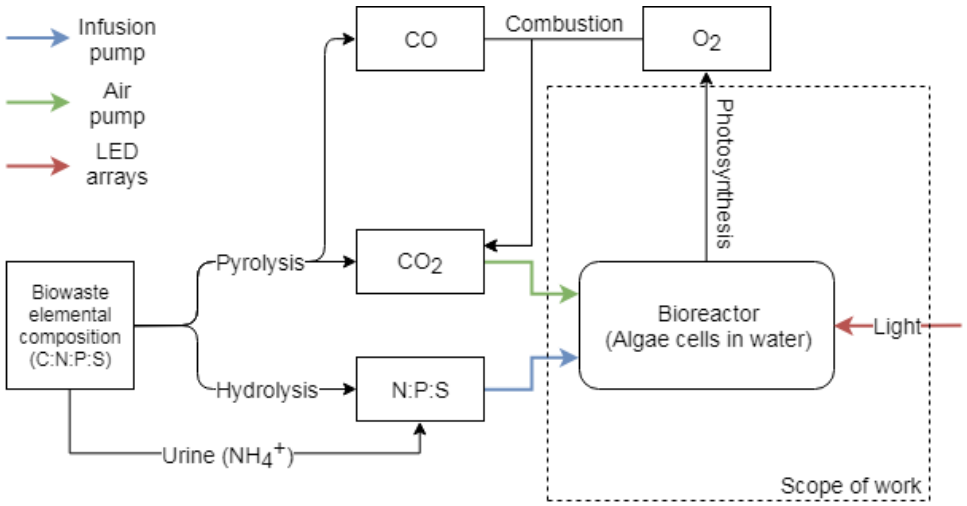


Figure 3.2: Function Structure Diagram with interfaces and defined scope of work: Construction of bioreactor and subsystems for inputs (nutrients and light)

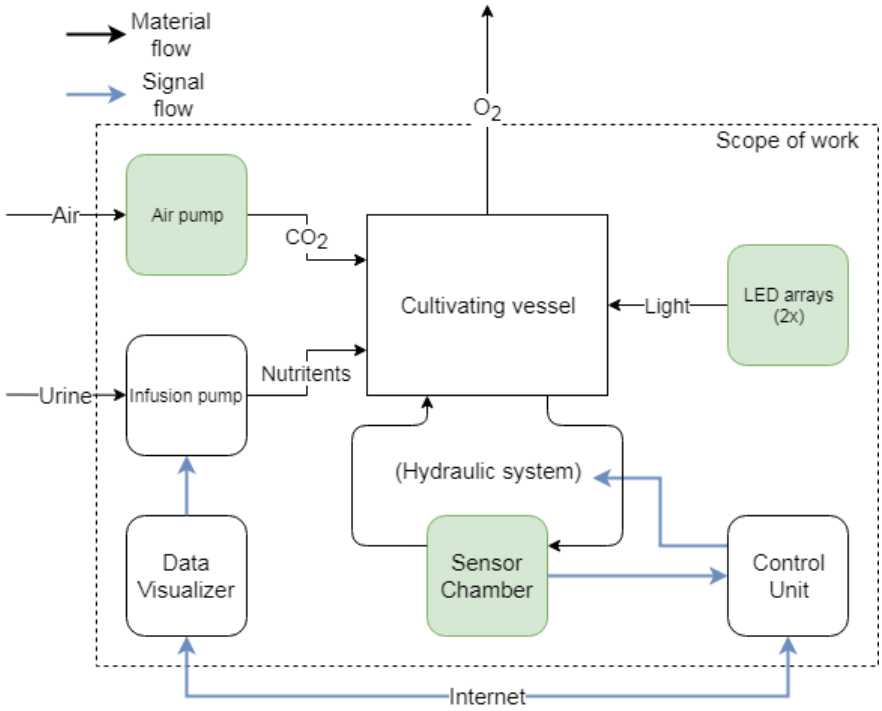


Figure 3.3: System diagram: (green) existed, ready-to-integrate components; (white): need-to-realize components

3.2 Cultivating vessel

The cultivating vessel is made of acrylic, as known as Plexiglas or Poly(methyl methacrylate, PMMA). The material is a transparent thermoplastic, commonly available in sheet form, and is often used as a lightweight or shatter-resistant alternative to glass. It is also bio-compatible.

- PMMA weighs about 49% of borosilicate glass ($\lambda_{PMMA} = 1180 kg/m^3$ [13] and $\lambda_{glass} = 2400 kg/m^3$ [14])
- PMMA also offers better thermal insulation ($c_p = 0.187 - 0.209 W/mK$) than glass ($c_p = 1.10 W/mK$)
- Deciding factor is the optical clarity: Glass has 90% light transmission, for acrylic it's 92%.

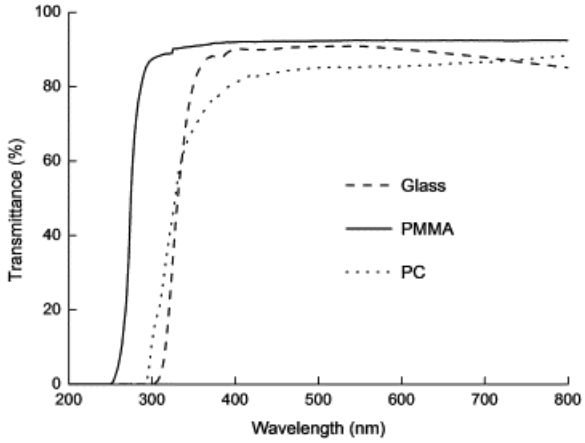


Figure 3.4: UV–vis spectra for Pyrex glass, PMMA, and PC substrates. [15]

To minimize the manufacturing cost, the reactor tank is dimensioned as follow:

The body consists of a wall fusing with the bottom lid. The wall comprises two 6 mm thick front and back plates, homogeneous fused with two 15 mm thick (composite) side plates. The bottom lid is made of two 5 mm thick plates, sealing the tank off (See Appendix B.1). The volume inside the tank is L 640 mm x W 35 mm x H 500 mm, gives enough space for 11.12 liters of algal medium.

When containing the maximum possible medium (11.12 Liters), the walls and tank floor are subjected to the hydrostatic pressure of 0.5 m of water. The pressure p acts on the floor is $p = \rho * gh = 0.05 bar$ [1], results an uniformly

[1] h: maximum static water head

distributed load on the bottom lid (The resultant force $F_R = p * A_{floor} = 112N$). The contact area between the lid and four vertical walls ($A = 158cm^2$) is filled with 2K-glue, fusing the tank body permanent together. At the top, the tank is seal off with a top lid, clamped together by two L-shape aluminum profiles. A safety analysis and estimation of maximum deflection can be found in Appendix A.2. The maximum deflection (experimental) is $1.5mm$ in the centroid of the front and back plate.

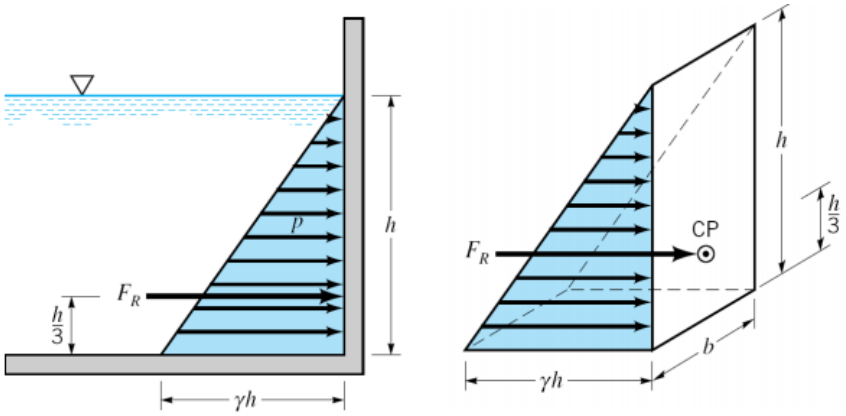


Figure 3.5: Hydrostatic pressure applied on the wall of the algal container without lid.

F_R Resultant force on the plate (N)

ρ density of medium (assumed to be = ρ_{water})

γ specific weight of the medium (= $\rho_{water} * g$)

b Width of the plate

CP Centroid of the plate

The reactor inner volume is connected to outside via three hollowed couplings (see Figure 3.6) and three solid couplings (see Figure 3.7). The outer couplings (top and bottom) are the inlet and outlet of liquid algal medium. These are connected with pipe/tube system with Festo connectors (Outside diameter: 6 to 6 mm). The middle coupling is planned for a relief valve, in case, the tank needs to work under compressed air/high pressure operations[1]. Every coupling carries two sets of O-ring (Nitrile rubber (NBR), ⌀ 8 mm) to seal and isolate the algal medium inside the tank and outside environment.

[1]the pressure inside the tank should never exceed 1.2 bars, to maintain the structural integrity of the vessel and the watertightness of the inner volume.

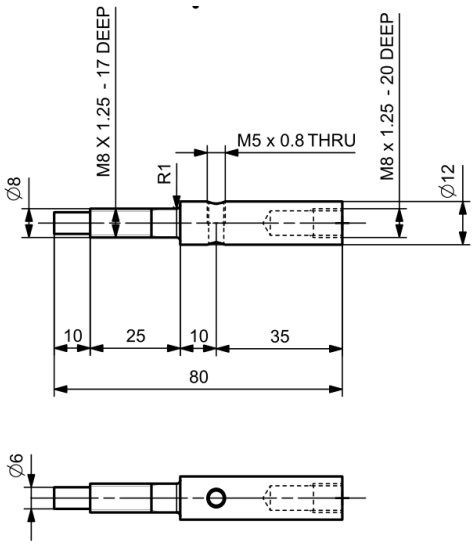


Figure 3.6: Solid coupling, used to carry the guiding rod for baffles

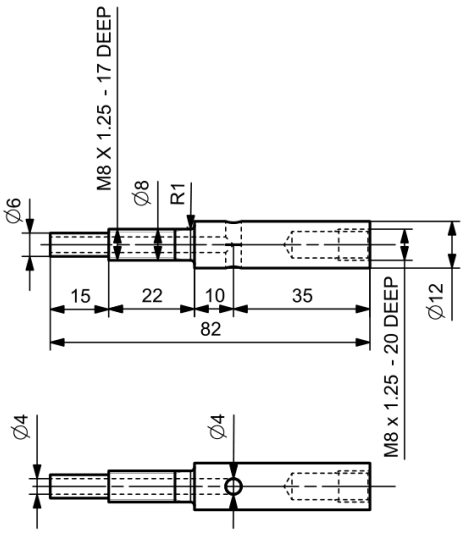


Figure 3.7: Hollowed coupling, used as inlet and outlet for algal medium

The inlet and outlet has an openings with a diameter of 4 mm, which would allow a flow rate (*Note: the flow will be reduced with reduced height*):

$$V_{max} = C_d * A * (\sqrt{2 * g * H}) = 0.000022 m^3/s = 1.32 L/min$$

Where:

$C_d = C_c C_v$ is discharge coefficient (= $0.62 * 0.97 = 0.6$)

C_c contraction coefficient (sharp edge 0.62, well rounded 0.97)

C_v velocity coefficient (water 0.97)

A area of aperture - flow outlet (= $1.257x10^{-5} m^2$)

H static water head for 10 liters (= $0.45m$)

g acceleration of gravity (9.81 m/s^2)

The opening inside the tank allows a left-over volume of about 100 mL. This will help keeping any unwanted debris from getting into the sensor chamber and the pump system.

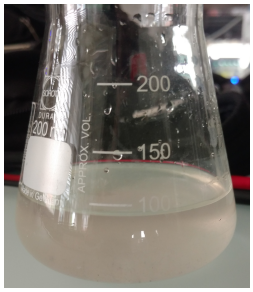


Figure 3.8: Left-over liquid medium, after completely draining the main tank

Aeration subsystem

At the bottom of the tank, an aeration device in form of membrane tube (*AirPrax* of BubbleTech) is implemented. This design is chosen, because of its economical as well as technical advantages over other possible designs:

- The pore diameter of the membrane tube is uniform, and therefore outputs same size microbubbles consistently along the length of the tube. Airstones are made of porous rock with opening in various sizes, which lead to an inconsistency of bubble's size. They also have tendency of forming fouling on the surface or breaking off when impacted by mechanical shock or high pressure.
- In comparison with ceramic plates/domes, the tube costs significantly less, as it requires no custom-made design.

For the experimenting purpose, the bubbles' size is important. The micro-sized bubbles (range from 10-100 μm) are required, to improve the mixing process and more efficiently exchange of gases.

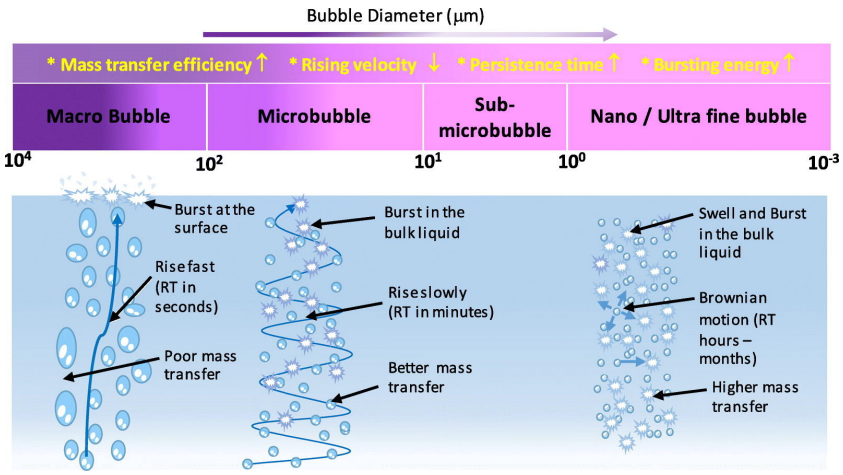


Figure 3.9: Proposed range of the bubble sizes and major properties. [16]

The tube ($L = 50cm$) requires a supply of compressed air at low-positive pressure (0.35 bar), to output microsized bubbles through 30'000 openings. It's made of EPDMX rubber, which is bio-compatible.

Baffle system

With the goal of impeding the air flow velocity, rising from the aeration device, multiple baffles are put inside of the tank (see Figure 3.10). However, the microsized bubble have the tendency of coalescing together in all viscous liquids , such as olive oil, dilute salt solutions, H_2SO_4 and distilled or tap water[17]. However in aqueous solutions of alcohols, organic acids, ether, benzene, concentrated HNO_3 and strong salt solutions, such as sea water[1], the bubbles would not recombine (the transition from coalescence to non-coalescence is 8 to 10 g salt/L).

During the experimental phase, the bubbles couldn't rise properly and stuck on the bottom surface of the baffles. To aid the rising of bubbles, the baffles are set at angle of 10 degrees from the horizontal plane.

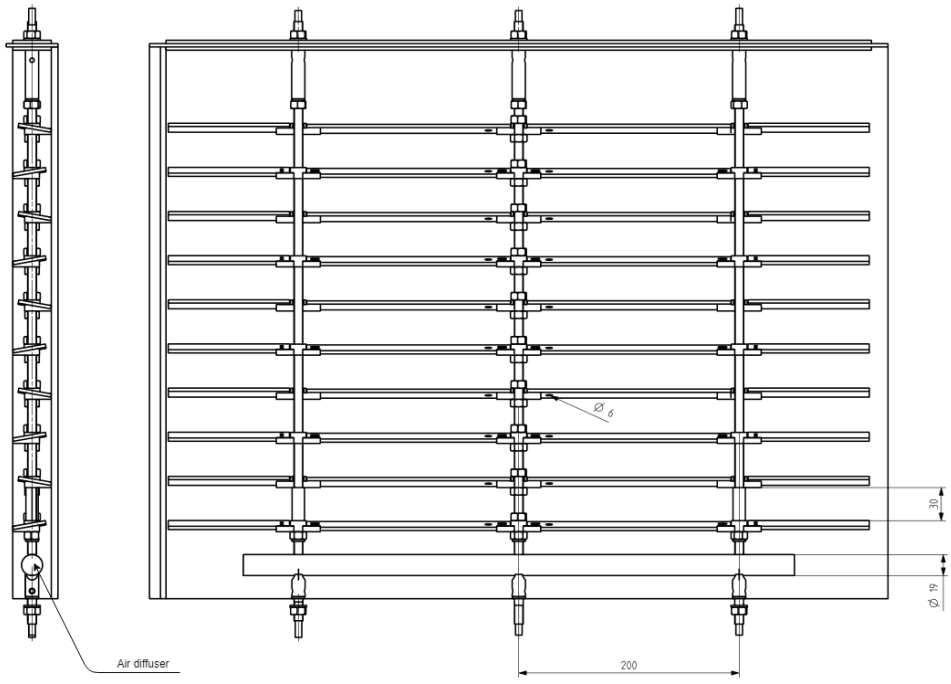


Figure 3.10: Baffle construction schema

Other solution was using a special coating solution, to make the baffle bot-

[1]Emiliania huxleyi is an oceanic algal typus, and requires sea water (> 35 g salt/L) to thrive

tom surfaces hydrophillic (see 3.11). The bubbles would not stay in one place and would glide upwards. However, due to the risk of bio-incomparability and the cost factor, this design solution was not implemented.

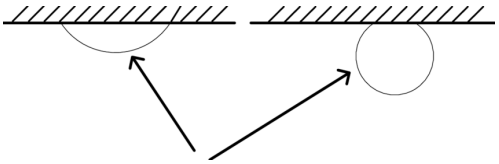


Figure 3.11: Bubbles on hydrophilic and hydrophobic surfaces.[18]

Every level of the baffles is made of four separated plates, connected and hold in plate with three holders. These holders are 3d-printed with PLA, and easy to clip on the guiding rods (polyamide PA66). On the center guiding rod, the baffle holders are kept in place by a set of two PA66 nuts, with constant distance of 3 cm between them.

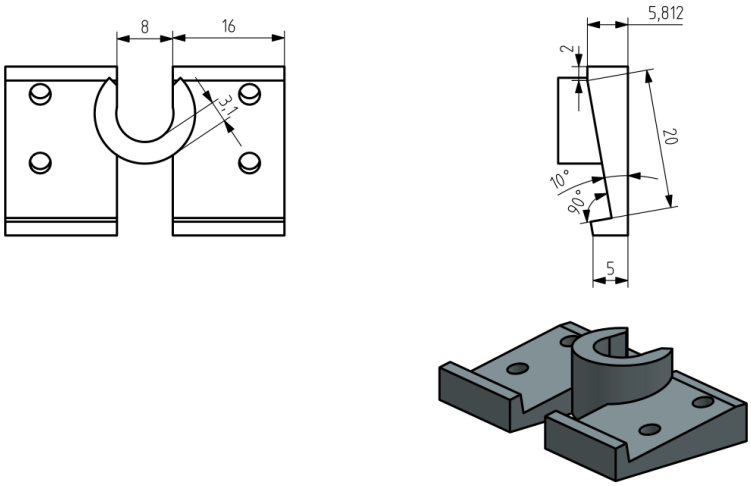


Figure 3.12: A 3D-printed baffle holder

3.3 Supporting frame

The cultivating tank and its ancillary system are screw-connected onto an aluminum modular assembly system of Kanya AG via two M8x40 bolts (Steel Grade 8.8) on each side. Each bolt (Shear capacity = 16 kN) can carry main tank with all subsystems included ($m_{total} \approx 12kg$) alone. However, the acrylic plate could be irreparably damaged if mounted incorrectly (Minimum requirement: One bolt on each side, and a third bolt to prevent unwanted rolling).

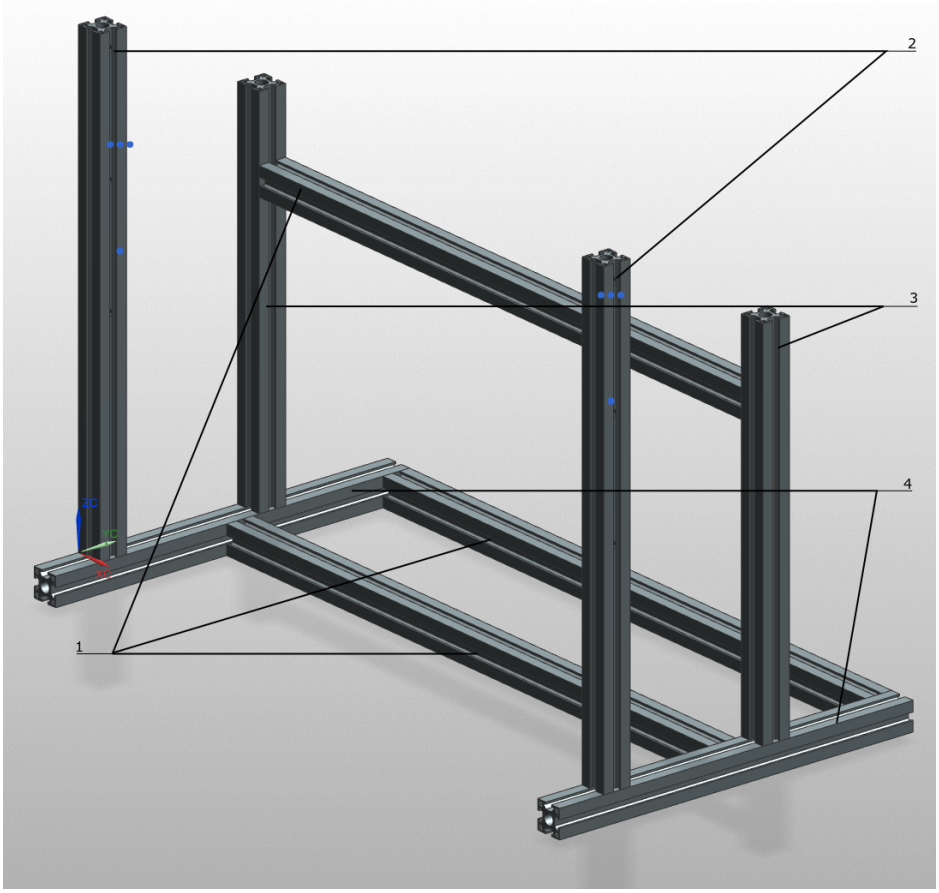


Figure 3.13: Frame assembly

Table 3.1: Bill of material (BOM) for supporting frame

Pos	Description	Amount
1	Four sided lightweight extrusion 30 x 30 x 670 mm	3
2	Four sided lightweight extrusion 30 x 30 x 500 mm	2
3	Four sided lightweight extrusion 30 x 30 x 400 mm	2
4	Four sided lightweight extrusion 30 x 30 x 450 mm	2
5	Standard connectors with electrical bonding	10
6	T-bolts, 8.8 steel, zinc coated	6
7	End caps 30 x 30 mm	8

The LED arrays can be mounted onto part (1) between parts (3) with T-bolts (6). By releasing the connectors between part (3)(4) and moving parts (3) horizontally, the distance from the array to the reactor can be adjusted.

Similarly, release the connectors between part (1)(3) and moving part (1) vertically, the height of the LED array can changed, to ensure a complete illumination of reactor. A small bubble level (or spirit level) is installed in the middle of LED-carrying beam to ensure that the beam is leveled.

3.4 Hydraulic and pneumatic system

3.4.1 Hydraulic system

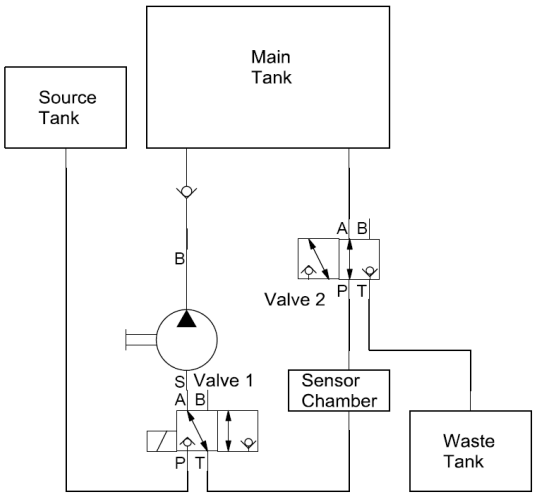


Figure 3.14: Schema of the hydraulic loop of the bioreactor

To further support and improve the agitation process, a liquid circulation subsystem is implemented. This system (see Figure 3.14) consists of:

- Three containers: Medium, source tank (15 Liters), flat plate, main cultivating tank (10 Liters) and waste product tank (15 Liters).
- Two directional control proportional valves of Bürkert type 0127, orifice size: 1.6 mm: one normally closed (NC, **Valve C**), and one normally open (NO, **Valve D**) are used to control the flow of the algal medium. The original valve type 6012 with brass housing is not suitable for sea water operations. Other possibility is use housing (armature guide tube included) with stainless steel or any alloy from 1.45xx. However, Bürkert don't have any option with this alloy, type 0127 (rocker valve) is chosen.
- One diaphragm pump of KNF Flodos NF 1.60 DCB with a nominal flow rate of 0.65 l min^{-1}. This rate is controllable by pulse width modulation (PWM, 0-5V) of the diaphragm frequency.

- Piping system uses one standard diameter size ($\varnothing 6mm$). The pipes, or tubes, is made of Polyurethane, are chemical, hydrolysis resistant, food-safe and transparent (to help checking whether bubbles enter the sensor loop.
- All fittings are from Festo (QS-Type). For water applications, fittings (NPKA-Type) are preferred but not required.

Procedure

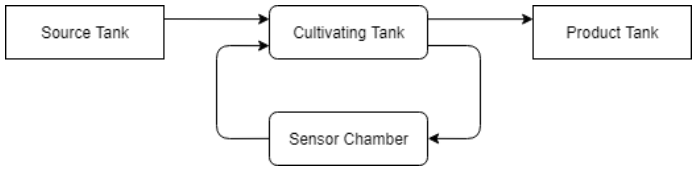


Figure 3.15: Operation loop of reactor

The reactor receives feed from the source tank, then detaches itself from the open loop (Source Tank - Cultivating Tank - Product Tank). The algal medium will be circulated in the closed loop (Cultivating Tank - Sensor chamber) during the whole experiment duration for set duration [1]. This helps provide sufficient agitation by cycling the algal culture through the reactor periodically. However, the primary mixing will be accomplished using bubbled gas to induce vertical circulation and easy shedding. Circulation keep microalgae cells well mixed so they receive the high illumination and homogeneous nutrient concentrations that they require to grow. At the end of the experiment, the main vessel will re-attach to the open loop, and drain the algal medium to the product tank. Then, the experiment can be restarted if needed.

The silicone tubing is connected and secured with the components using Festo couplings [19], to prevent them from slipping during operation. Due to the near-incompressibility of algal medium, the usage condition differs from the generally applicable pneumatic technical data for these coupling. Adherence to the parameters (pressure and temperature) stated below is absolutely essential for conducting experiments safely.

- Pressure range [bar]: 1 - 6 bar (Peaks must be within specified range)
- Temperature range [°C]: 1 - 50 °C
- Medium: water with no harmful additives.

[1] For IGLUNA experiment, the duration for the closed-loop (Cultivating Tank- Sensor chamber) is about one week

3.4.2 Pneumatic system

The pneumatic system relies on an air pump (EHEIM 400), which can output 0-400 Liters of air per Minute at 1 bar.

3.5 Sensor system

3.5.1 Sensors

The key parameters, to be monitored during experiments, are temperature, pH value, dissolved oxygen (DO), chlorophyll, fluorescence and the optical density (OD) of the culture.

- Temperature sensors: submersible probes of Dallas Semiconductor (DS18B20). The probes are installed at following locations:
  - Main cultivating tank
  - Sensor chamber (to calibrate pH sensor [1])
  - External (to measure atmosphere temperature). This is required, to intervene, when the heating system of glacier dome fails.
  - Electrical box (to monitor the wasted heat output by PSU)
- The pH value and the dissolved oxygen are measured using commercial probes from Gravity.
- The optical density and chlorophyll count are monitored using two photodiodes (one behind the long-pass filter) with an absorption bandwidth of $350 - 1100nm$. The diode detects the light, emitted by the LED ($430nm$ and $750nm$), scattering by the algae. This implementation is based on the measurement concept (see Figure 2.4).

All measuring probes were calibrated before being implemented into the system (B.3). These measurements are recorded using a Raspberry Pi. The process can be controlled manually or automatically following a schedule, using `cron`. *Cron* is a Linux utility which schedules a command or script on the micro-controller to run automatically at a specified time and date.

[1] In certain solutions, the pH of the solution will change (typically increase) as the solution cools.

3.5.2 Sensor chamber

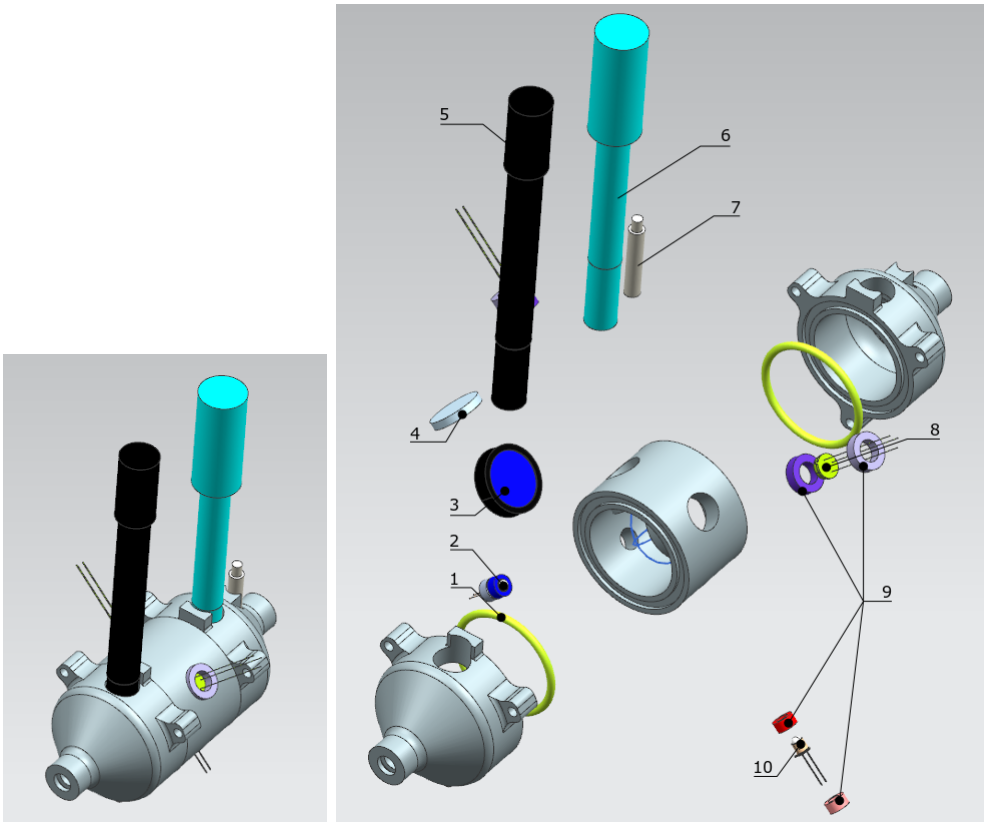


Figure 3.16: Sensor chamber: assembled (left) and exploded view (right)

1. O-ring (⌀ 44 mm)
2. LED ($\lambda \approx 430nm, I_{max} = 100mA$)
3. Long-pass filter $\lambda \approx 650nm$
4. Transparent len.
5. Dissolved O_2 sensor
6. pH probe
7. Temperature sensor
8. Photo-diode $\lambda \approx 350 - 1100nm$
9. Concentric rings, to ensure the LED and the photo-diode lined up correctly.
10. LED ($\lambda \approx 750nm, I_{max} = 100mA$)

Various methods have been used to monitor the gaseous species (O_2, CO_2) involved during the cultivation process. In this design, the measurements follow the conventional method, using probes *in situ*. The housing chamber is 3D-printed with PLA (Polylactic Acid) as material. PLA and its copolymers are commonly used for a wide variety of applications, and are considered to be bio-compatible. Upon bio-degradation by special bacteria, the polymer begins breaking down, usually by hydrolysis, into lactic acid (LA) or to carbon dioxide and water. The median half-life of the polymer is 30

weeks [20]. For longer experimenting duration, ABS (Acrylonitrile Butadiene Styrene) is a viable alternative. However, algal medium has no known effects on PLA.

During the experimental phase, it's clear that the sensor chamber is the weak point of the system. Two important problems are:

1. Minor leaking during long-termed operation.
2. Probe mounting positioning and the potential of vortex-induced vibration (VIV)

Leakage

The chamber can stay leak-free under static condition. However, while in operating condition, there is minor leaking through the sensor mountings. For long-term, autonomous operations without on-site monitoring, this is required a new redesign. Due to the material as well as geometry of the chamber wall, a possible design for the sensor mounting is proposed as follow:

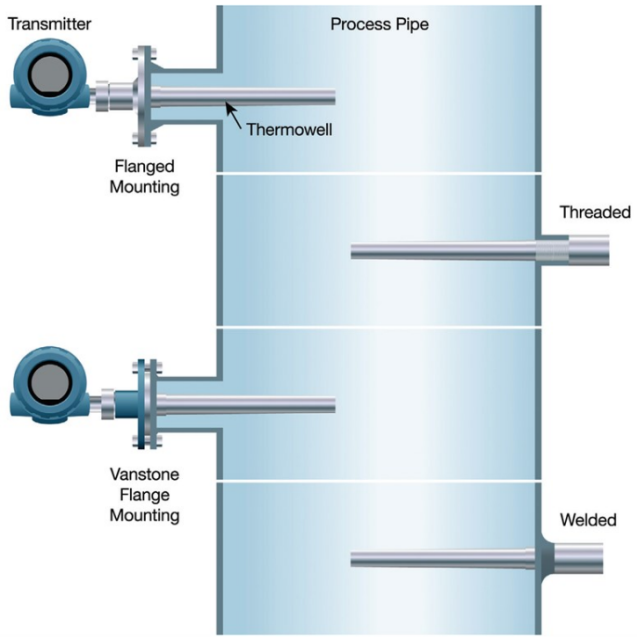


Figure 3.17: Variety of other approaches: Threaded is the most simple method, which requires the least design change. However, the 3D-printing as manufacturing method is not recommended. Molded or machined plastics (Duroplaste such as POM-C (Acetal)) is prefered.

Another approach is using standard solutions from Georg Fischer (GF). They offere a wide selection of installation fittings that can control the position of

the sensor probes in relation to the dimensions of the pipe-liked chamber section.

Type	Description
Plastic tees	• 0.5 to 2 inch versions • MPVC or CPVC
PVC Glue-on Saddles	• Available in 10 and 12 inch sizes only • Cut 2-1/2 inch hole in pipe • Weld in place using solvent cement
PVC Clamp-on Saddles	• 2 to 4 inch, cut 1-7/16 inch hole in pipe • 6 to 8 inch, cut 2-1/8 inch hole in pipe
Iron Strap-on saddles	• 2 to 4 inch, cut 1-7/16 inch hole in pipe • Over 4 inch, cut 2-1/8 inch hole in pipe • Special order 14 in. to 36 in.

Type	Description
Iron, Carbon Steel, 316 SS Threaded tees	• 0.5 to 2 in. versions • Mounts on threaded pipe ends
Carbon steel & stainless steel Weld-on Weldolets	• 2 to 4 inch, cut 1-7/16 inch hole in pipe • Over 4 inch, cut 2-1/8 inch hole in pipe
Fiberglass tees FPT	• 1.5 in. to 2 in. PVDF insert
Union Fittings and Wafers	• For pipes from DN 15 to 50 mm • PP or PVDF

Figure 3.18: Georg Fischer option for pipe fittings

Probe mounting

Besides the leaking issue, additional considerations about mounting positions of probes such as temperature sensors with thermowell [1] or pH need to be taken. The general rule is locating the sensor in a trap or where the flow is upward helps to protect the sensor from exposure to air bubbles when the system is in operation. For this reason, the chamber should be positioned horizontally, below the outlet of the cultivating tank or have its inlet at lower level than the outlet.

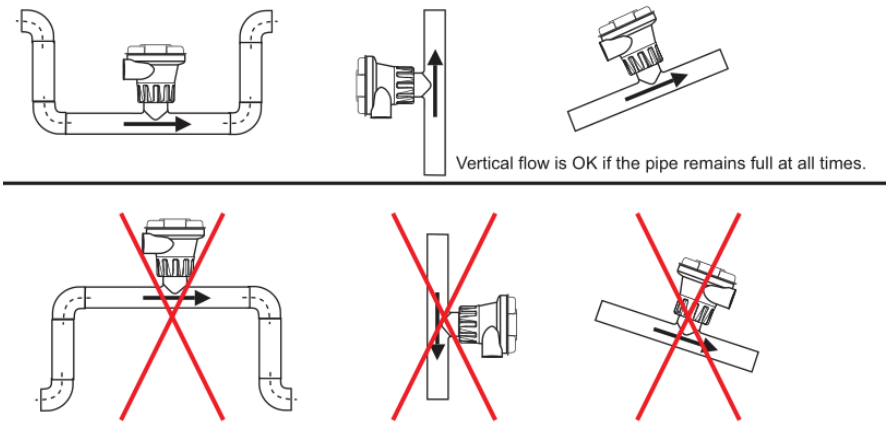


Figure 3.19: The lower configurations are to be avoided, as it's difficult to keep the pipe full.

The current design with all the probes mounted in a single line also intro-

[1] Thermowell is cylindrical fittings used to protect temperature sensors installed in industrial processes.

duces a new issue: Vortex-Induced Vibration (VIV). When any sensor is inserted into moving liquid, the flow can produce hydrostatic and aerodynamic forces around that sensor. Under certain conditions, the fluid that flows around the cylindrical probe creates a wake. This wake generates vortices that rotate in opposite directions and then detach (shed). The phenomenon is called the Kármán vortex street.

Vortex shedding creates a periodic lift force normal to the direction of the flow and a periodic drag force inline with the flow. These forces cause the probe to shake. This is the root cause that cause material fatigue at the connection between the probe and chamber wall. This leads to previously mentioned issue, leakage during operation. The American Society of Mechanical Engineers (ASME) provides a method for finding the necessary dimension for a thermowell to be strong enough to operate satisfactorily in a given application (see [21]).

3.6 Electrical system

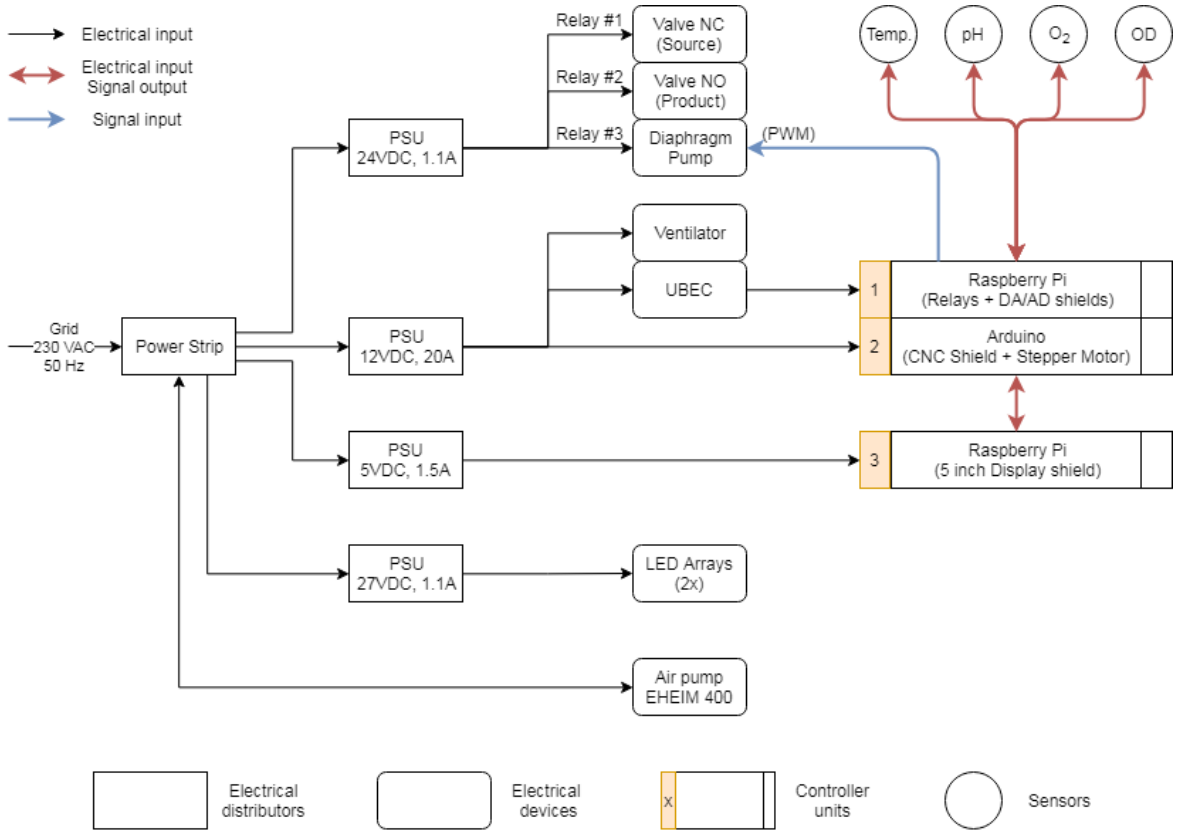


Figure 3.20: Electrical schematic

Electrical switchboard

A polycarbonate enclosure (L 300 mm x W 230 mm x H 111 mm) is used to contain:

- The Raspberry Pi is powered by 5 volts direct current (VDC), converted from a 12 VDC via an universal battery eliminator circuit (UBEC), also known as a step-down converter. It carries a 3-canal relay board (solenoid valves are wired to NC, pump to NO) and a AD/DA Expansion board (using ADS1256, 30ksps sampling rate)
- An Arduino board and a CNC-Shield, is powered by 12VDC, drawn directly from a PSU outside of the box.
- A laboratory PSU (Delta Elektronika ES150) draws power directly from the grid via the power strip. Its output is set at 27VDC, 1.1A, to power the LED arrays.
- A solid-state drive SSD for easy-access to datalogs.

To avoid overheating, a ventilator is built in the enclosure. The air flows from outside to inside, causes a pressure differential (Δ_p (inside - outside) > 0). Since the pressure inside is higher, the air will flow out through any opening, and lower the dust accumulation inside.

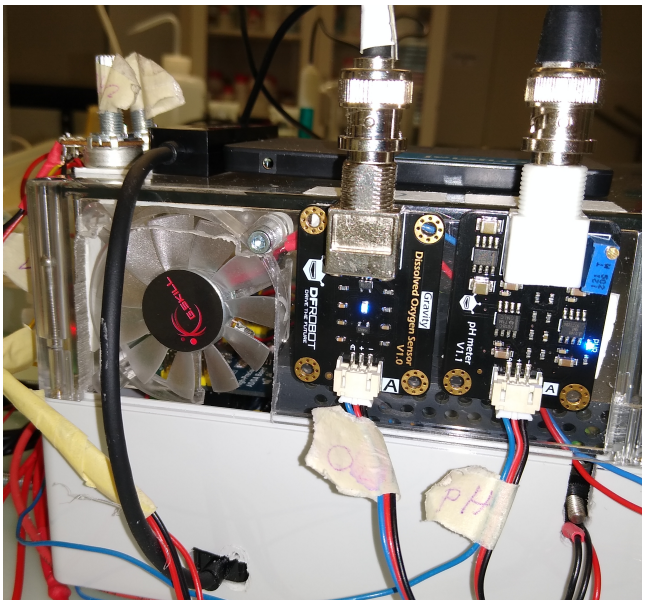


Figure 3.21: Ventilator mounting and connection port for pH and O_2-sensor. The SDD (black) is mounted on top of the enclosure

Total power usage is 64.75 Watts at nominal level, with a peak at 72.75 Watts

during the transition phase from the open operation loop to the closed loop (see Figure 3.15). For detailed consumption, see Appendix A.5.

3.7 Infusion system

A proven, existing solution of PatcherLab (CalTech), named *Poseidon* was chosen to be implemented into the reactor's ecosystem, to improve the capabilities of the system. The *Poseidon* syringe pump system is an open source alternative, which is low-cost and easy to assemble, and also easy to scale up/add more identical units. It uses 3D printed parts and common components that can be found at any Do-It-Yourself (DIY) retailers. The pump control can be run cross-platform (Windows, Mac, Linux), or directly over a Raspberry Pi computer with GUI.

Table 3.2: Bill of material (BOM) for one infusion pump

Pos	Description	Amount
1	NEMA 17 Servo motor 1.8 degree, 1.2A	1
2	5mm to 5mm Motor Shaft Coupling	1
3	6mm steel rod ($L = 200mm$)	2
4	M5x0.8 threaded rod ($L = 170mm$)	1
5	M5x0.8 nut	1
6	M3x0.8 bolts and nuts (for mounting motor)	4
7	Arduino + CNC Shield Pack	1

This BOM is derived from the original BOM, listed by PatcherLab (with linear bearing, and M5 knob omitted). This should lower the difficulty of sourcing the necessary materials. The design is also adjusted accordingly to keep fulfilling the functionality of the subsystem.

- To make it easier to print with smaller printer, the pump base (green, see Figure 3.22) is separated in the middle in two parts. Then these parts are connected together with two M3 threaded pins ($L = 10mm$).

- The openings, in which previously a linear bearing has to be inserted are made smaller. The pump carriage will slide directly on two smooth 6mm steel bolts (3)

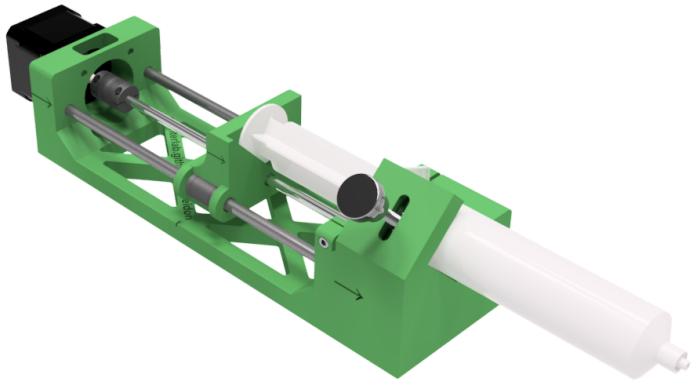


Figure 3.22: The originial design

The software [1] is configured to control the stepper motor with 200 steps per revolution at up to 1/32 microstepping. That translates to maximum 6400 steps per revolution. A flow rate reference table for various microstep settings and spring sizes can be found in the appendix (A.4).

For each cycle of experiment (seven days), the syringe ($25mL$) will be replaced or refilled with nutrients (N:P:S). The nutrient will be injected directly intro the loop (Cultivating Tank - Sensor Chamber)[2]

[1] https://github.com/patcherlab/poseidon

[2] For IGLUNA project, due to time limitation, the Source Tank will be pre-mixed with nutrients. The infusion pump system is not activated during the whole demonstration.

Chapter 4

Programming

4.1 Procedure and known issues

Operation Procedure

The bioreactor is required to operate continuously during the experimenting duration. For the IGLUNA-Project, the experiment will take place from June 23, 2019 to June 30, 2019 (One cycle over a week)[2].

At the start of the experiment, the system will read the signal from two NO Reed-switches (both are `OFF`)[3] to confirm the empty state of the cultivating vessel. The valve C will turn `ON` (Valve D stays `OFF`). After that the diaphram pump will turn on for $750s$ [4] or until the lower Reed-switch turns to `ON`. This continues for another $750s$ or until both switches are `ON`.

Now the system will be ready for growing algae: Valve C will turn `OFF`, and a timer (TOF) for 7 days ($604800s$) will count down to the end of the cycle. During this time, the algal medium will be circulated through the sensor chamber. Once per minute, the sensors will read and log the measurements.

Once the cycle is completed, the system will turn valve D `ON` to drain out 5 Liters of the growed algae to a product container. The program will repeat over.

[2]The system can operate infinitely, as long as there is no power/mechanical failures
[3]the float switches is not yet implemented, (May 31, 2019)
[4]$t = 750s$: measured time to pump 5 Liters into cultivating vessel

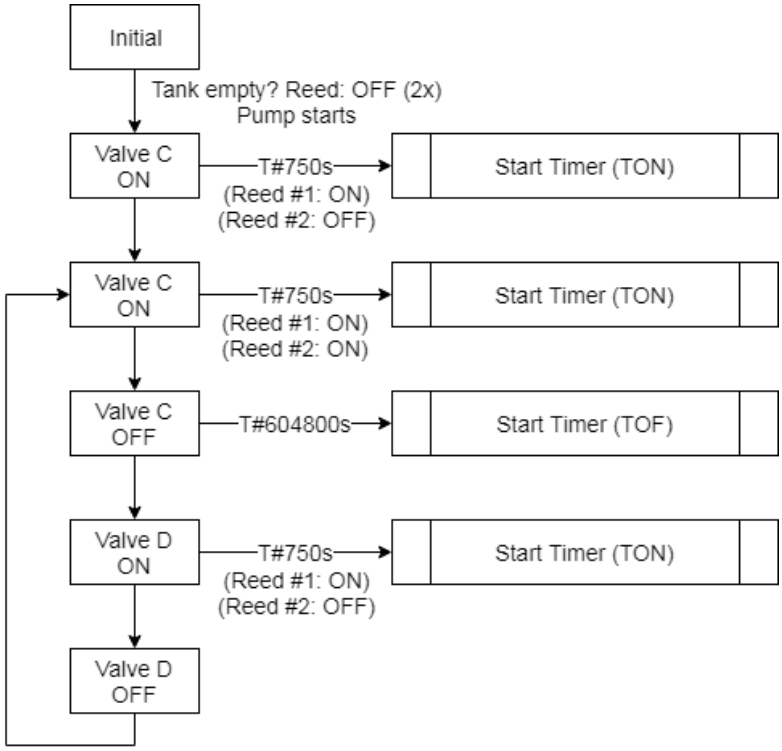


Figure 4.1: Sequential function chart (SFC) for the bioreactor

During the experiment cycle (see Figure 3.15), the controller needs to control two solenoid valves (for flow direction), and read data from the sensor array. Due to time limitations, the control software has been only developed to a rudimentary level. At the time of writing, a number of issues hasn't been mitigated. There is a temporary solution for each problem, described below, as well as proposal for further development in the future.

Known issues

1. System has no realtime clock (RTC). It requires internet connection to update and keep track of time.
   Proposal: Implement a RTC chip, powered by a coin battery: PCF8523 is inexpensive, DS1307 is most common, and DS3231 is most precise.
2. Water level control: The cultivating tank is outfitted with two Reed-switches at marking position (5 Liters and 10 Liters). However, there are no floats with magnet implemented yet. The system relies on time interval to operate. This can not ensure a high precision operation, if there is minor change in the enviroment or system.
   Proposal: There are several methods to sense the water level:

Table 4.1: Types of water level sensors [22]

Type	Pro	Con
Optical	Compact, no moving parts, high pressure and temperature capability, can detect tiny amounts of liquids	Invasive as the sensor requires contact with the liquid, requires power, certain thick substances can cause coating on the prism.
Vibrating or tuning fork	Compact, cost effective	Invasive, number of uses are limited
Ultrasonic	No moving parts, compact, reliable, not affected by media properties	expensive, invasive, performance can be affected by various elements in the environment
Capacitance	Solid-state, can be non-invasive, compact, accurate	May require calibration, can only be used in certain liquids
Radar	very accurate, no calibration required, multiple output options	expensive, can be affected by the environment, limited detection range
Conductivity or resistance	No moving parts, easy to use, low-cost	Invasive, liquids need to be conductive, probe erosion
Float (preferred solution)	Non-powered, direct indication, relatively inexpensive, various outputs	Invasive, moving parts, large in size, large amount of liquid has to be present before the float makes contact.

3. The scripts is clustered, with each has its dedicated function. A treemap chart is required to help users locate the correct script.

Software Requirements

Raspberry Pi run on a modified Debian-Linux enviroment (**Raspbian Stretch 2019-04-08**, for:

- Infusion Pump system: Arduino IDE, AccelStepper, OpenCV and Qt5 [1]

- Sensor arrays and valve control: BCM2835 Library [1]
- 5-inch LCD: LCD-show Library [2]

4.2 Scripts

- The scripts for IGLUNA can be found under: `$HOME/IGLUNA`.
- The valve and pump control is at: `$HOME/IGLUNA/RPi_Relay_Board`
- The sensor main file (each type of sensor can be found in a child folder at this location): `$HOME/IGLUNA/Sensors`

pH	`$HOME/IGLUNA/Sensors/00_pH`
Temperature	`$HOME/IGLUNA/Sensors/01_Temp`
Dissolved O_2	`$HOME/IGLUNA/Sensors/02_O2`
Optical density	`$HOME/IGLUNA/Sensors/03_OD`

Task scheduling

To run a command any specified time, edit the crontab by `crontab -e`, and add following code:

```
# * * * * *  command to execute
#
# | | | | |
# | | | | |
# | | | | .--- day of week (0 - 7) (0 to 6 Sunday to Saturday)
# | | | .--------- month (1 - 12)
# | | .------------- day of month (1 - 31)
# | .----------------- hour (0 - 23)
# .----------------------- min (0 - 59)
```

For example, this will start all the sensors every 5 minutes, and the output will be appended to the `datalog.txt`

```
*/5 * * * * $HOME/IGLUNA/Sensors/python3 start-sensors.py \
>> datalog.txt
```

Another option is run the command at constant interval using `sleep` command:

[1] http://www.airspayce.com/mikem/bcm2835/
[2] https://github.com/waveshare/LCD-show

```
while true; do $HOME/IGLUNA/Sensors/python3 start-sensors.py;\
sleep 300; done
```

To help users set `cronjob` up, Raspian has `gnome-schedule` as an GUI option. An alternative is using a web service: https://crontab-generator.org/

Valve and pump control

The valves are set to `OFF` during normal operation (to spare energy) and only turn to `ON` during feeding and draining operation. Valve D (Channel CH1), Valve C (Channel 2)

The pump is set up to be always `ON`, and can be turn `OFF` for emergency. Pump (Channel CH3)

To manually change the state of the relays, for example to turn off the pump:

```
sudo $HOME/IGLUNA/RPi_Relay_Board/Relay.sh CH3 OFF
```

Infusion pump control

To start the control software of the infusion system, connect the USB cable with the controller:

Figure 4.2: Controller: Raspberry Pi + 5 inch Display

The software can be started by:

```
$HOME/IGLUNA/poseidon/SOFTWARE/python3 poseidon_main.py
```

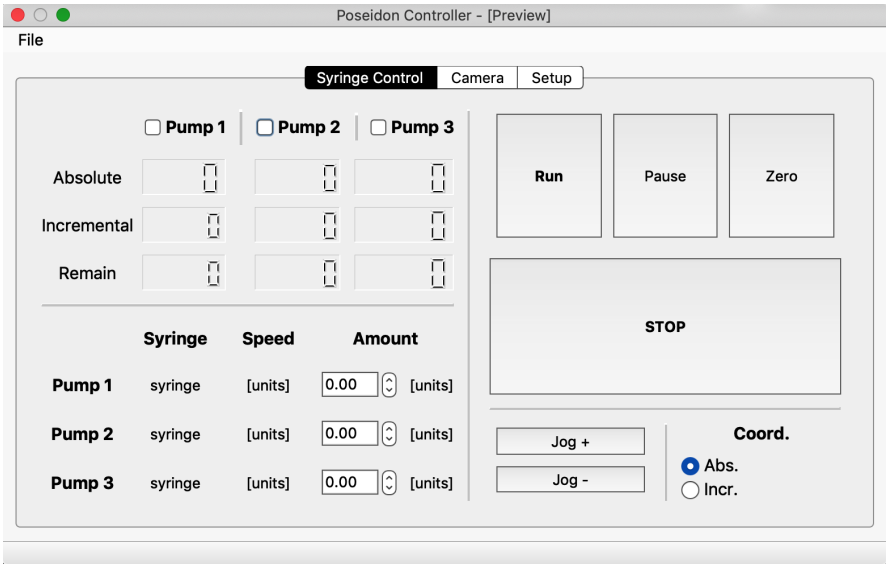


Figure 4.3: Control software for infusion syringe pump, given by PatcherLab, CalTech. Currently, only pump #3 is available

Chapter 5

Summary

5.1 Current status

At the time of this writing (May 31, 2019), the concept is nearly realized as a functional prototype with about 85% of all defined tasks are completed.

- Implement a system of baffles to improve the mixing process inside the reactor (by generating vortices). [done]
- Build a gas/nutrient infusion to systematically and periodically saturate the algal medium. [done]
- Integrate the bioreactor into the system, and set up for manual operations (with lighting, and hydraulic circulation) [done]
- Choosing and adjust design to fit the aeration device [done]
- Design and construct an electrical switchboard, which contains the control system and power supply. [done]
- Program the control software for automated operation. [done]
- Trial run the system in laboratory before delivery. [planned]

With the current design focus on flexibility and modularity, the system can be adjusted and modified for various possible experiment parameters. However, as discussed in 3.5.2, the sensor chamber requires a new design, to be fully released as a working product. During the trial run, the system functions as per requirements while the measurement readings form the sensor chamber are unreliable[2].

It's however not yet determined whether Raspberry Pi is sufficient and reliable for operations in extreme environment. It's advised to consider replacing it with an industrial-grade Programmable Logic Controller (PLC) system or a Programmable Relays (Zen Series by OMRON). For more budget

[2] pH value isn't correct and have the tendency of drifting.

approach, micro PLC based on Raspberry Pi (such as PiXtend), which can be programmed with SFC, Ladder diagram (LD), Function block diagram per IEC 61131-3 via CodeSys, is a viable option.

5.2 Next steps

The prototype will be tested in the next two weeks (June 7, 2019 to June 21, 2019). After that, the whole system will be taken apart to Ready-To-Assemble state, pack up and send to the demonstration event at the Glacier Palace, Zermatt, Wallis, Switzerland on June 23, 2019. There it will be installed and set to run autonomously during the event until June 30, 2019. The cultivated algae will be harvested and, in the laboratory in Hergiswil, the biomolecules will be extracted and processed. The complete system will be transported back for further experiments in the future.

Appendix A

Calculation/Assessment/Calibration

A.1 Morphological table

Reactor form	Flatplate air lift reactor	Annular column	Tubular reactors		
Baffle construction (to avoid bubble coalesence)	Special coating	Angling baffles			
Aeration devices	Airstone	Membrane tubes	Ceramic plates		
Pump art	Gear	Piston	Vane	Membrane (Diaphragm)	Peristaltic
Flow direction control	Multiple 2/2 valve (on/off)	2x 3/2 valves (1x NO, 1x NC)			
Controller	Arduino	Raspberry Pi	Beaglebone		
Mixer	Air lift (air diffuser on the bottom)	Motorised mixer	Shaker	Utilising pump's pulsation	
Remote control	Wifi	GSM			
Data visualization	Local (LCD screen)	Website (hosted on controller)			

Figure A.1: Morphological table was used to help decide the design decisions. Green indicates the chosen solutions

A.2 Minimum wall thickness

The front and back plates are affected mostly from the hydrostatic pressure, due to the longer span (in comparison with the shorter span of side plates). To select a suitable thickness, a short calculation is provided below to roughly estimate the minimum thickness as well as the expected maximum displacement. For detailed and more exact calculations, a FEM analysis is required.

To approach this problem mathematically, there are three options:

- Westergaard plate theory, with the assumption that Poisson's ratio $\nu = 0$. This was used to size the wall thickness.
- Kirchhoff-Love plate theory, with the assumption that Poisson's ratio $\nu = 0.3$ (most materials have this ratio, PMMA has a ratio of 0.37). This was used to estimate the maximum wall deflection, under hydrostatic pressure.
- Timoshenko Theory of plates and shells, which is valid for all ν. This was for reference only.

A.2.1 Westergaard approximate solution [1]

Acording to Westergaard, the stress is always greater in the shorter span (GH) than in the longer span (EF). The deflections of the two strips at the center of the plate are equal, however, the shorter strip has a smaller radius of curvature.

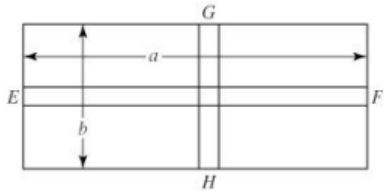


Figure A.2: Longitudinal (EF) and transverse (GH) plate strips

This approach requires defining a working stress limit. With the yield strength of PMMA, $\sigma_{yield} = 45.0MPa$, the working stress limit is set at 33.3% the given yield strength as safety measure ($\sigma_w = 15MPa$).

For the plate's area (L 640 mm x H 500 mm, H/L = 0.78 $\approx$ 0.8) and the mean pressure on the plate $p = \rho * g * H = 1000 * 0.5 * 9.81 \approx 5000Pa$, the bending moment coefficient can be read the following diagram (with the experimental value):

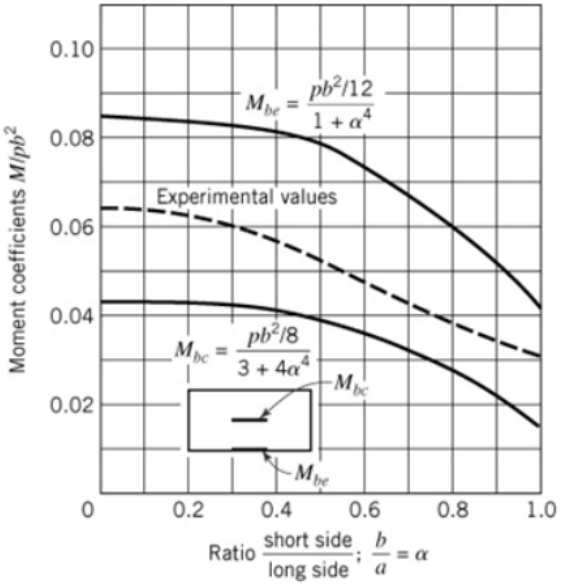


Figure A.3: Ratio of bending moment M in rectangular plates with fixed edges, under hydrostatic pressure.

The bending moment:

$$M = 0.038 * p * L^2 = 0.038 * 5000 * 0.64^2 = 77.8\ Mm/m$$

As:

$$\sigma = \frac{M_z * y}{I_z} = \frac{M_z}{W_z} = \frac{6 * M}{h^2} \Rightarrow h = \sqrt{\frac{6 * M}{\sigma}} = \sqrt{\frac{6 * 77.8}{15}} = 5.57mm$$

Based on this result, the tank thickness should be at least 6 mm, to be both safe and economically viable.

A.2.2 Kirchhoff-Love's theory

According to Kirchhoff, the stress resultants (considering moment equilibrium with respect to x, y, z axes):

$$\frac{\delta^2 M_x}{\delta x^2} + 2\frac{\delta^2 M_{xy}}{\delta x \delta y} + \frac{\delta^2 M_y}{\delta y^2} = p(x, y)$$

In differential form with respect to the displacement ω, the definition of the moment component (M_x, M_y and M_z) is subtituted with the stress-displacement relations:

$$M_x = -D_k(\frac{\delta^2\omega}{\delta x^2} + \nu\frac{\delta^2\omega}{\delta y^2}) \text{ and } M_y = -D_k(\frac{\delta^2\omega}{\delta y^2} + \nu\frac{\delta^2\omega}{\delta x^2})$$

$M_{xy} = -D_k(1-\nu)\frac{\delta^2\omega}{\delta x \delta y}$ with $D_k = \frac{E}{1-\nu^2}\int_{-t/2}^{t/2} z^2 dz$ the bending rigidity of the plate

That gives:

$$\frac{\delta^4\omega}{\delta x^4} + 2\frac{\delta^4\omega}{\delta x^2 \delta y^2} + \frac{\delta^4\omega}{\delta y^4} = \frac{p(x,y)}{D_k}$$

The calculation of the integrals requires calculating the coefficient C_1, which is time-consuming. A calculator ([23]) computes the maximum displacement and stress of a clamped (fixed) rectangular plate under a triangular load.

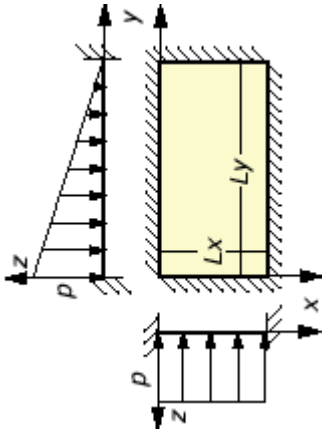


Figure A.4: Free body diagram with boundary conditions: All edges of the plate are clamped

Loading:	triangular load p =	5000	Pa
Geometry:	Width L_x =	0.64	m
	Length L_y =	0.5	
	Thickness h =	6	mm
Material:	Young's modulus E =	3.3	GPa
	Poisson's ratio ν =	0.3	
Output:	Unit of displacement w =	mm	
	Unit of stress σ =	MPa	

Figure A.5: Calculator inputs

The maximum displacement is $\omega_{max} = C_1 * \frac{pL_y^4}{Eh^3}$ and the bending stress is $\sigma_{max} = C_2 * \frac{pL_y^2}{h^2}$ where the values of C_1 and C_2 are determined by *polynomial least-square curve fitting*. These values are listed in the following table:

L_x/L_y	0.6	0.8	1.0	1.2	1.4	1.6	1.8	2.0
C_1	0.0016	0.0047	0.0074	0.0097	0.0113	0.0126	0.0133	0.0136
C_2	0.1304	0.1436	0.1686	0.1800	0.1845	0.1874	0.1902	0.1908

From here, using *linear interpolation*, to determine C_1 and C_2 for $L_x/L_y = 1.28$.

$$y = \frac{x_2 - x}{x_2 - x_1} y_1 + \frac{x_1 - x}{x_1 - x_2} y_2$$

With $C_1 = 0.01034$ and $C_2 = 0.1818$, the $\omega_{max} = 4.55mm$ and $\sigma_{max} = 6.32MPa$

A.2.3 Timoshenko's theory

The deflection surface of a plate, clamped at four edges[24]:

$$\omega = \frac{2q_c H^4}{\pi^5 * D} \sum_{m=2,4,6,\ldots}^{\infty} \frac{(-1)^{m/2+1}}{m_5} (2 - \frac{2 + \alpha_m tanh\alpha_m}{cosh\alpha_m} cosh\frac{m\pi y}{H} + \frac{1}{cosh\alpha_m} \frac{m\pi y}{H} sinh\frac{m\pi y}{H}) sin\frac{m\pi x}{H} \quad \text{(A.1)}$$

A.3 Safety Assessment Report

Safety Assessment Report

Risk ID	Risk description	Current risk level (probability A-E)	Note
1	*Algal medium is frozen or chilled at below ideal temperature range (15°C-28°C)*	IV - D	**Reason:** Insulation prepared by P07 is in-sufficient, or heating is turned off over night. **Migitation:** Thermal jacket (2nd compartement) for cultivating tank (avoid anti-freeze agent), Heat pack (PCM) activate before closing time of each day. (10 liters medium should keep temp. above 10°C for several hours, due to good thermal mass).
2	*Cultivating tank - hydraulic connections leaks over time*	III - D	**Reason:** Festo couplings is usually used for pneumatic connections **Migitation:** operate system in design range (https://www.festo.com/wiki/en/Tubing_and_fittings_for_water); water leak storage implemented at corners of the reactor to collect any leaking (avoid spilling over the whole area)
3	*Overheating (LED array)*	II - E	**Reason:** too high electrical input **Migitation:** LED array feeds off a lab power supply unit (U: 27 V, I: 1.1 A), the PSU is hide in an electrical box to avoid unwanted access. The arrays carry a dedicated cooling block with fan (forced convection)
4	*CO2 leaking*	II - E	**Reason:** - **Migitation:** Increasingly CO2 input will cause the pH value of algal medium change (more acidic). As soon as the pH value go past defined limits, a 2/2 valve will shut CO2 tank down, until it goes back to normal
5	*GSM connection loss*	IV - C	**Reason:** - **Migitation:** Using SSC hotspot
6	*Cell death (Pump shear)*	II - C	**Reason:** A peristatic pump is preferred to pump biological medium. Diaphragm pump can have high shear around the integral NRV in the pump **Migitation:** Lower the flow rate
7	*Settling of cells in dead zones (bottom tank)*	IV - A	**Reason:** Due to designated draining point, some portions of cells won't get agitated by air/CO2 bubbles or drained out between experimenting cycles. **Migitation:** Adding slopes and lower draining point to force cell flow.

Where:

Probability class	Description	Probability/frequency
A	*The accident is expected to occur*	*>1/10*
B	*The accident is likely to occur*	*1/100 - 1/10*
C	*The accident is likely to occur at some point*	*1/1000 - 1/100*
D	*Improbable, but the accident could occur at some point*	*1/1000000 - 1/1000*
E	*Unlikely, the accident could only happen in exceptional circumstances*	*<1/1000000*

Injury class	Definition (own and other's property damage and remediation costs)
I	Approximately the same cost as a total system loss
II	Significant loss
III	Limited loss
IV	Slight loss

A.4 Infusion pump reference table

Microstep	Syringe Size: 1 mL	3	5	10	20	30	60
1		624	2,084	4,065	5,887	10,261	13,179	19,946
2	Maximum	499	1,667	3,252	4,710	8,209	10,544	15,957
4	Flow Rate	250	834	1,626	2,355	4,104	5,272	7,978
8	[mL/hr]	125	417	813	1,177	2,052	2,636	3,989
16		62	208	406	589	1,026	1,318	1,995
32		31	104	203	294	513	659	997

Table 1. Maximum rates for given syringe and microstepping: Maximum flow rates [mL/hr] for a single pump for a given microstepping and Becton Dickinson (BD) syringe size. At lower flow rates, higher microstepping is desirable for a smoother flow.

Microstep	Steps per Revolution	Precision [μm]	Maximum Speed [mm/s]
1	200	4	10
2	400	2	8
4	800	1	4
8	1600	0.5	2
16	3200	0.25	1
32	6400	0.125	0.5

Table 2. Stepper motor performance: Precision was calculated based on the steps per revolution and the pitch of the lead screw (0.8 mm/revolution). The maximum speed was measured for the displacement of the carriage with an error of 5.5% $\pm$ 1.5% of the set flow rate.

Figure A.6: The speeds and flow rates of the system are limited by the microstepping and the syringe size used.

To calculate at the flow rate:

$$\dot{V} = v * A$$

Where:

$\dot{V}$ Flow rate (ml/s)

v Syring's plunger velocity (mm/s)

A Cross secional inside area of the syringe's barrel (mm^2)

A.5 Electricity consumption

Power requirement:

Voltage Input	(V) Output	Power	(W)	Usage per week (h)		Description
230	24		4		0.2	Bürkert 3/2 directional valve, 0127, NC
	24		4		0.2	Bürkert 3/2 directional valve, 0127, NO
	12		7.5		168	KNF, Diaphragm pump, NF 1.60
	12		0.75		168	Ventilator
	5		12.5		168	Raspberry Pi + various sensors
	5		10		42	Raspberry Pi + dsiplay
230	230		30		168	LED modules
230	230		4		168	Air pump
	Max.		72.75			
	Overhead security		33.3	[%]		
	Requirements		96.97575			

Figure A.7: Power consumption of each component/subsystem over a week

A.6 Sensor Calibration

Table A.1: Bill of material (BOM) of sensor arrays

Measurement	Sensor	Accuracy[1]	Measuring range[2]
Temperature	**Dallas Semiconductor** DS18B20	± 0.5 K	-10°to + 85°
Dissolved O_2	**Gravity** Dissolved O_2	± 0.1%	0-20 ppm
pH	**Gravity** pH meter	± 0.1%	1-14
Chlorophyll a	Emitter: LED430L	± 10 nm	430 nm
	Receiver: FDS100Si	0.65 $\frac{A}{W}$	350 nm - 1100 nm
	Longpass Filter: FEL0650	n/a	650 nm
Optical Density	Emitter: LED750L	± 20 nm	750 nm
	Receiver: FDS100Si	0.65 $\frac{A}{W}$	350 nm - 1100 nm

A.6.1 Temperature sensor

To calibrate the sensor, it has to be submersed in waterbaths at various temperature. After that, the probe will be insert into another water bath at specified temperature. The measurement value is taken to compare with the known temperature. This is now known as *measruing error*. The measurement deviation is assumed to be linear. Using this infomartion, the measuring error can be compensated using a linear equation:

$$T_{corrected} = m * T_{measured} + b \text{ with b: Intercept (or Offset); m: Slope}$$

Table A.2: Calibration data

	T1	T2	T3	T4
Slope	1.006	1.031	1.013	1.030
Offset	-0.399	-0.605	-0.219	-0.241

For a more accurate and precise approach, see "Absolute Calibration of DS18B20 Thermometers" by Robert Kandrsmith[3]

A.6.2 pH sensor

The pH sensor is inserted into three different solutions (manufactured by **Hamilton**), with specific pH-value (4.01±0.02, 7.00±0.02, 9.21±0.02) at two

[3] https://www.kandrsmith.org/RJS/Misc/Thermometers/absolute_ds18b20.html

different temperature[1]. With three reading points, a linear function is fitted: Slope = 8.83; Offset = -10.388

A.6.3 O_2 sensor

Between a fully saturated solution and a solution with no O_2, the O_2-concentration trend is linear. To determine this linear function, three reference solutions with various concentration of O_2 (0%, 50%, 100%) are measured by the sensor.

- 0%: Nitrogen is pumped into the water solution. Nitrogen N_2 combines with oxygen completely, leaves no free oxygen in the solution.
- 100%: Oxygen is pumped into the solution. It's important, that two solution have the same volume.
- 50%: Two solution is mixed together.

Slope = 1, offset = 0

A.6.4 OD sensor: Fluorescence and Chlorophyll a

The OD develops linear with the quantity of algae cells. Two mearsurements are made with salt water (no cells) and one solution with $898x10^9$ cells per Liter [2]. Use these two reading points, the correlation between measured voltage (from receiver) and the quantity of cells can be determined.

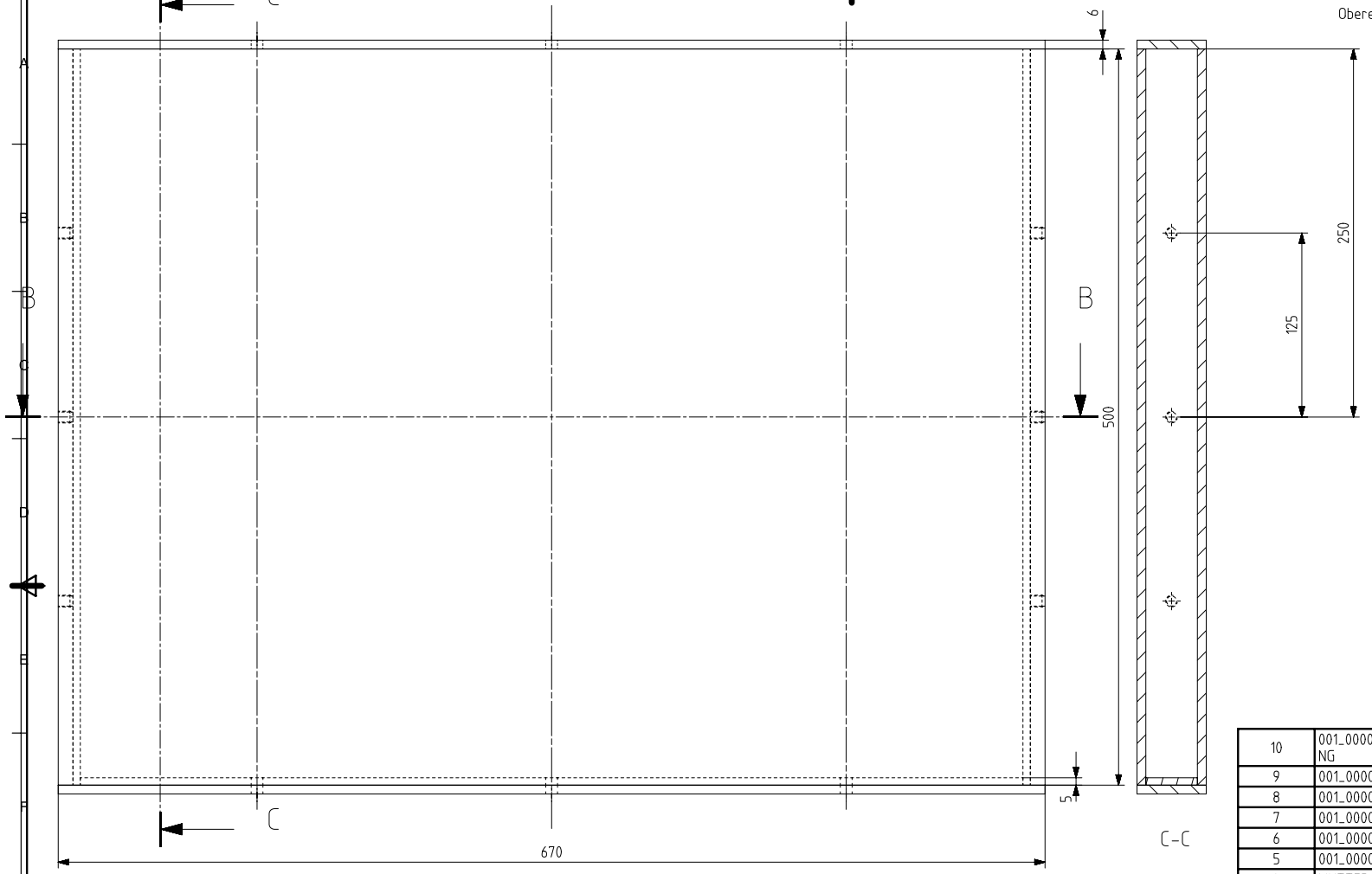
[1] this is to verify any measurement change due to temperature dependency of pH-values
[2] The cell quantity is counted by Cytometer BD Accuri C6 Plus

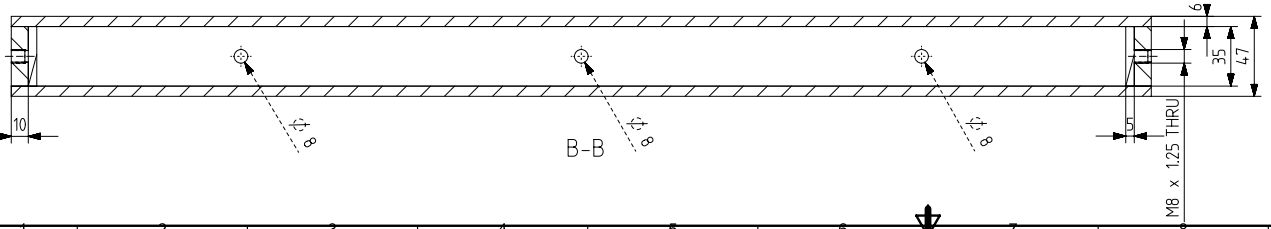
Appendix B

Drawings

B.1 Cultivating tank

Obere und untere Platte nicht zukleben.





PC NO	PART NAME	QTY
10	001_000001_00_M_TANK_HORIZONTAL_SLATE_SEALING	1
9	001_000001_00_M_TANK_BAFFLE	9
8	001_000001_00_M_TANK_VERTICAL_SLATE	2
7	001_000001_00_M_TANK_GUIDING_ROD	2
6	001_000001_00_M_TANK_HORIZONTAL_SLATE	2
5	001_000001_00_M_TANK_DRAINING_ROD	1
4	MUTTER_M8	6
3	SCHEIBE_M8	6
2	001_000001_00_M_TANK_VERTICAL_SIDESLATE_OUTSIDE	2
1	001_000001_00_M_TANK_VERTICAL_SIDESLATE_INSIDE	2

Aenderungsstand / change state
revision date name

Werkstoff / material
-

Behandlung Werkstoff / material treatement
-

Oberflaechen Behandlung / surface treatement
-

Mst. / scale
Format / size
A2
Einheit / unit
mm
Blatt / sheet
1/1

gezeichnet
gepruefft
freigegeben

Name / partname

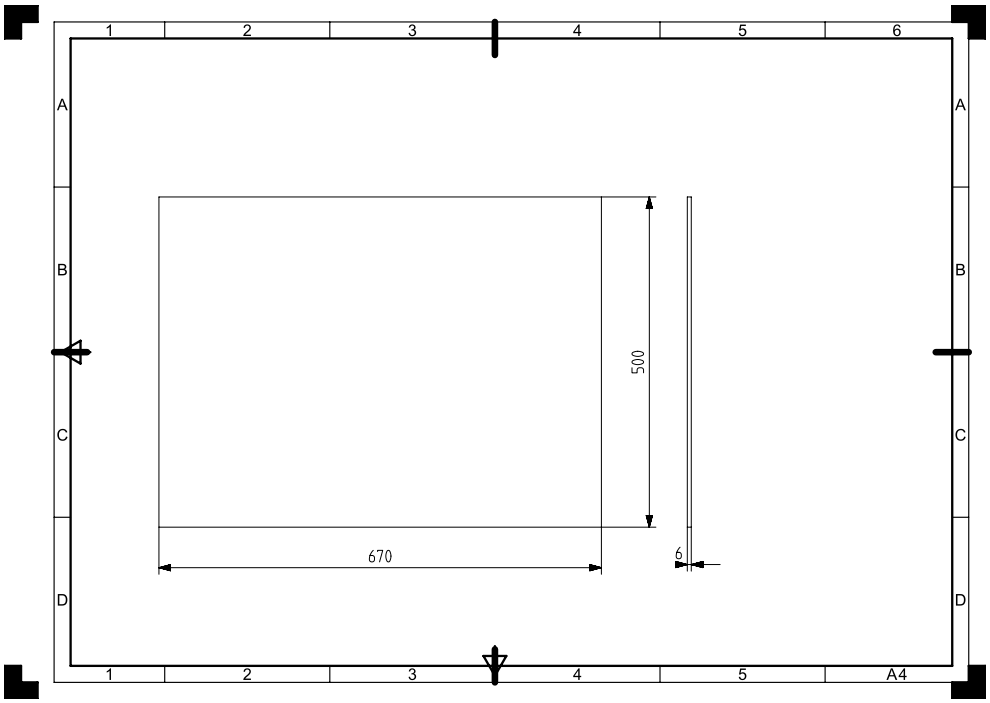
Projekt / project
rev.

Zeichnungsnummer / partnumber

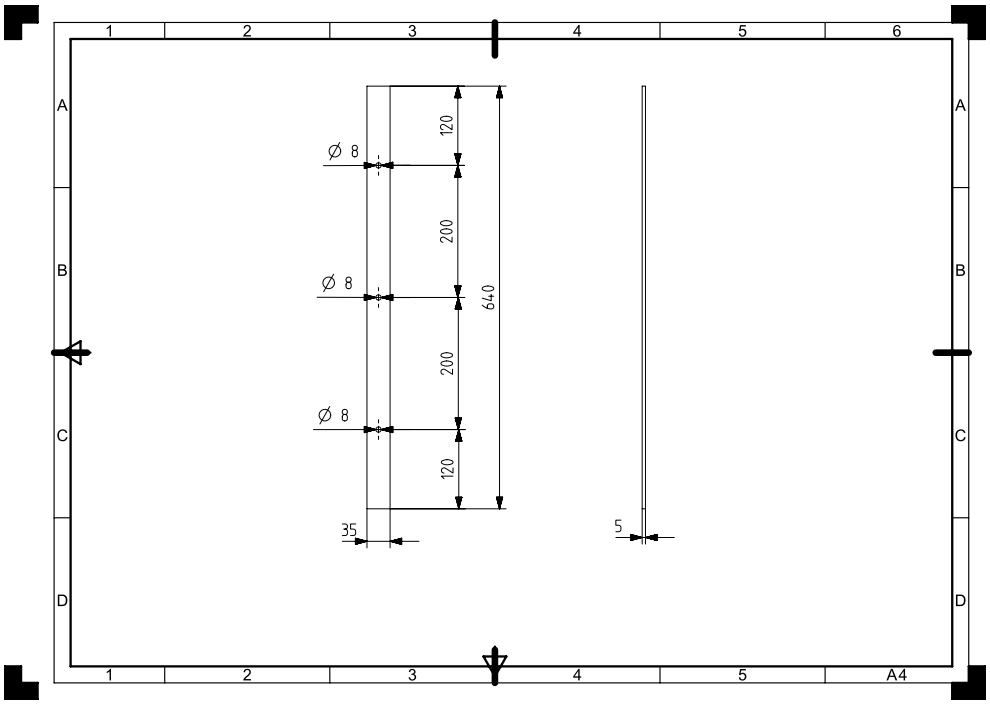
Dateiname / file name

A2

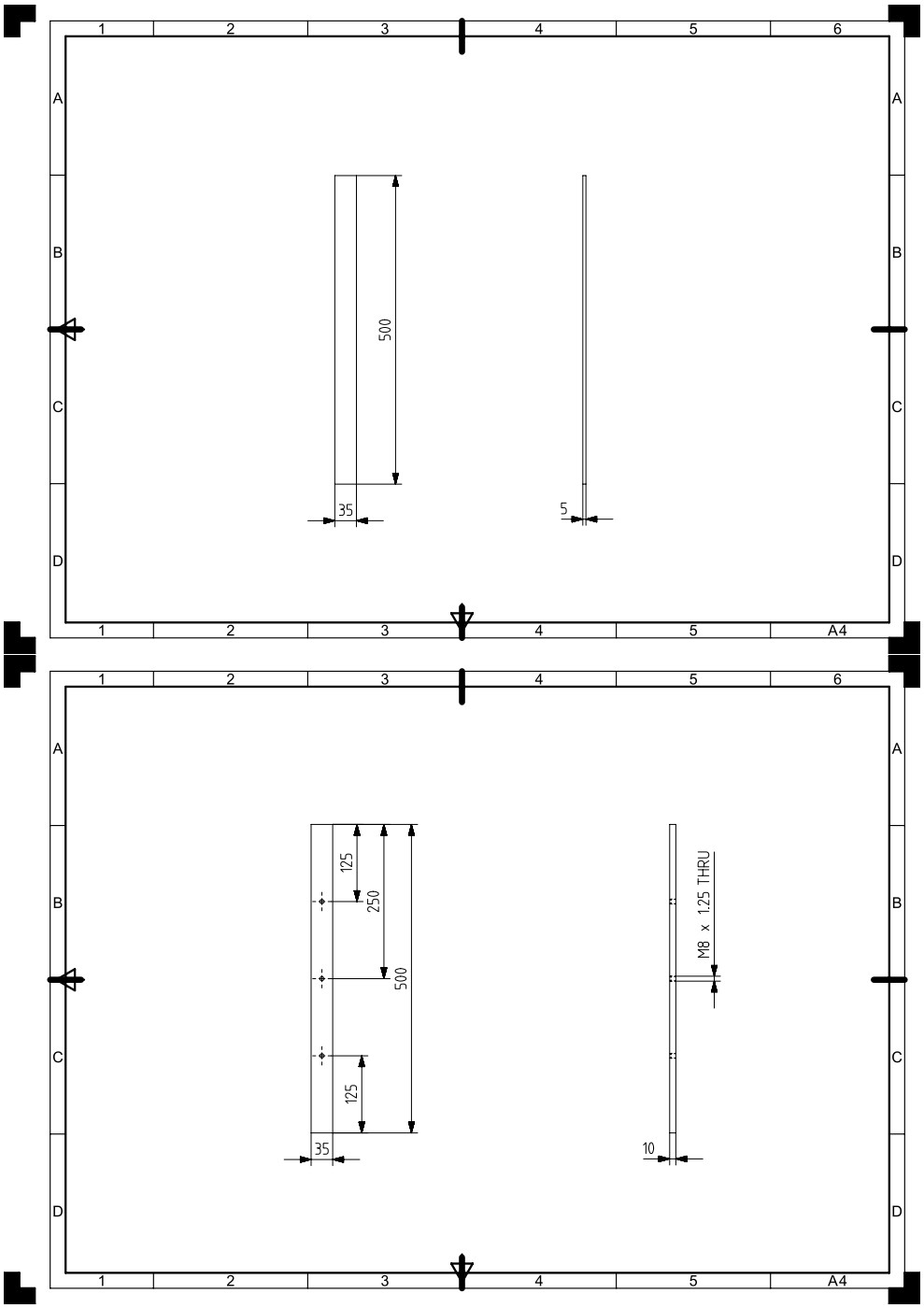
Front plate

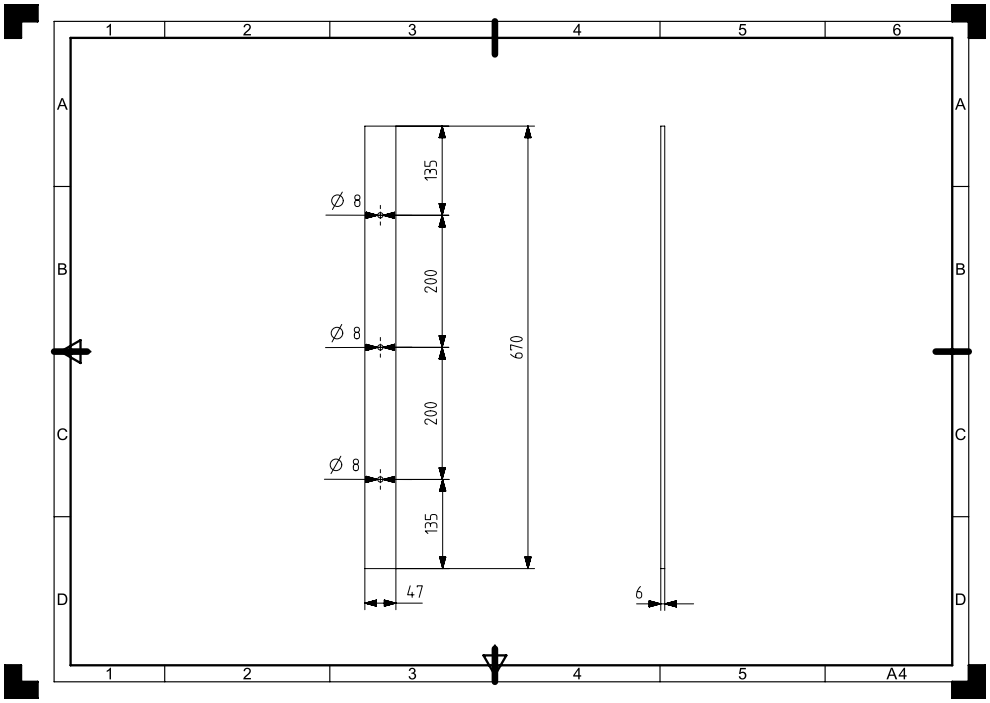


Bottom plate



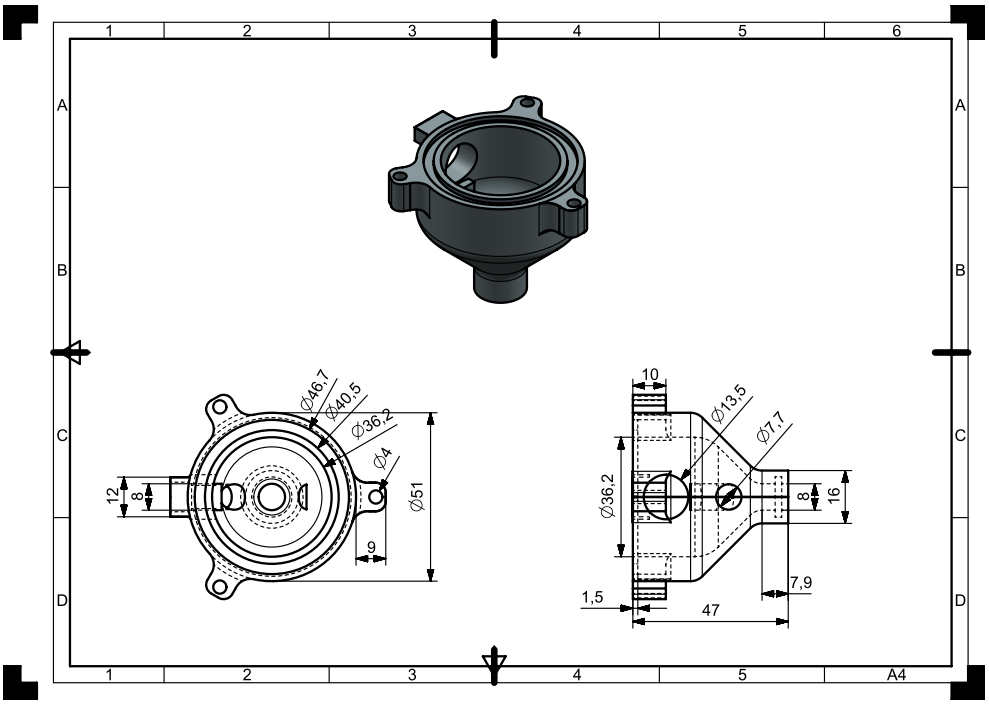
Inner side plate



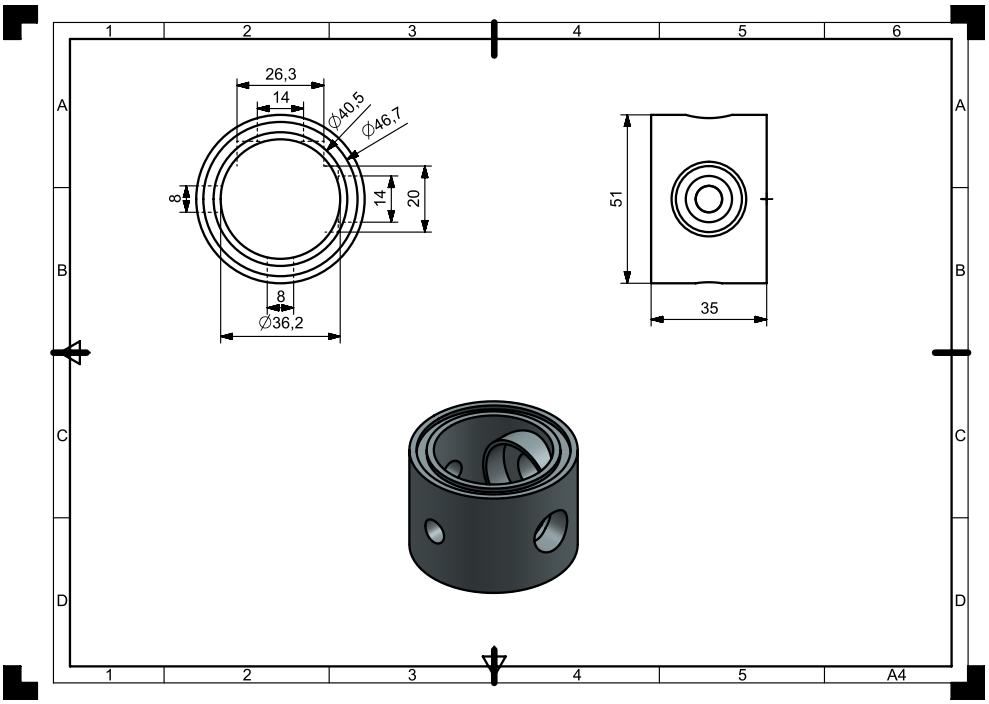

1
2
3
4
5
6
A
B
C
D
Ø 8
Ø 8
Ø 8
135
200
200
135
670
47
6
A4

B.2 Sensor chamber

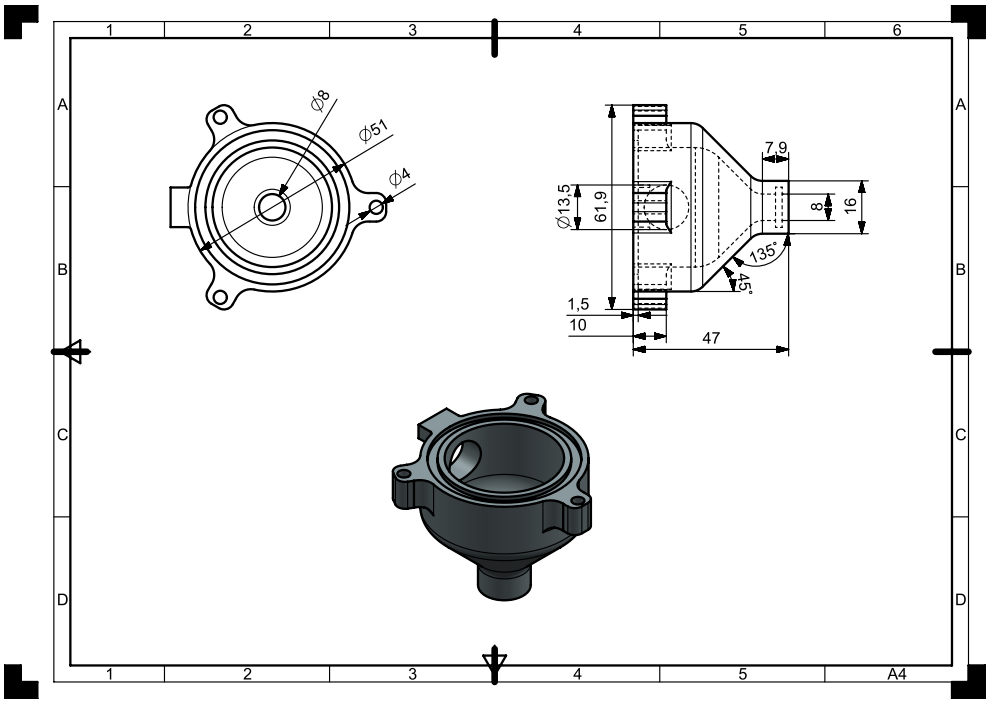
1. Top part of chamber



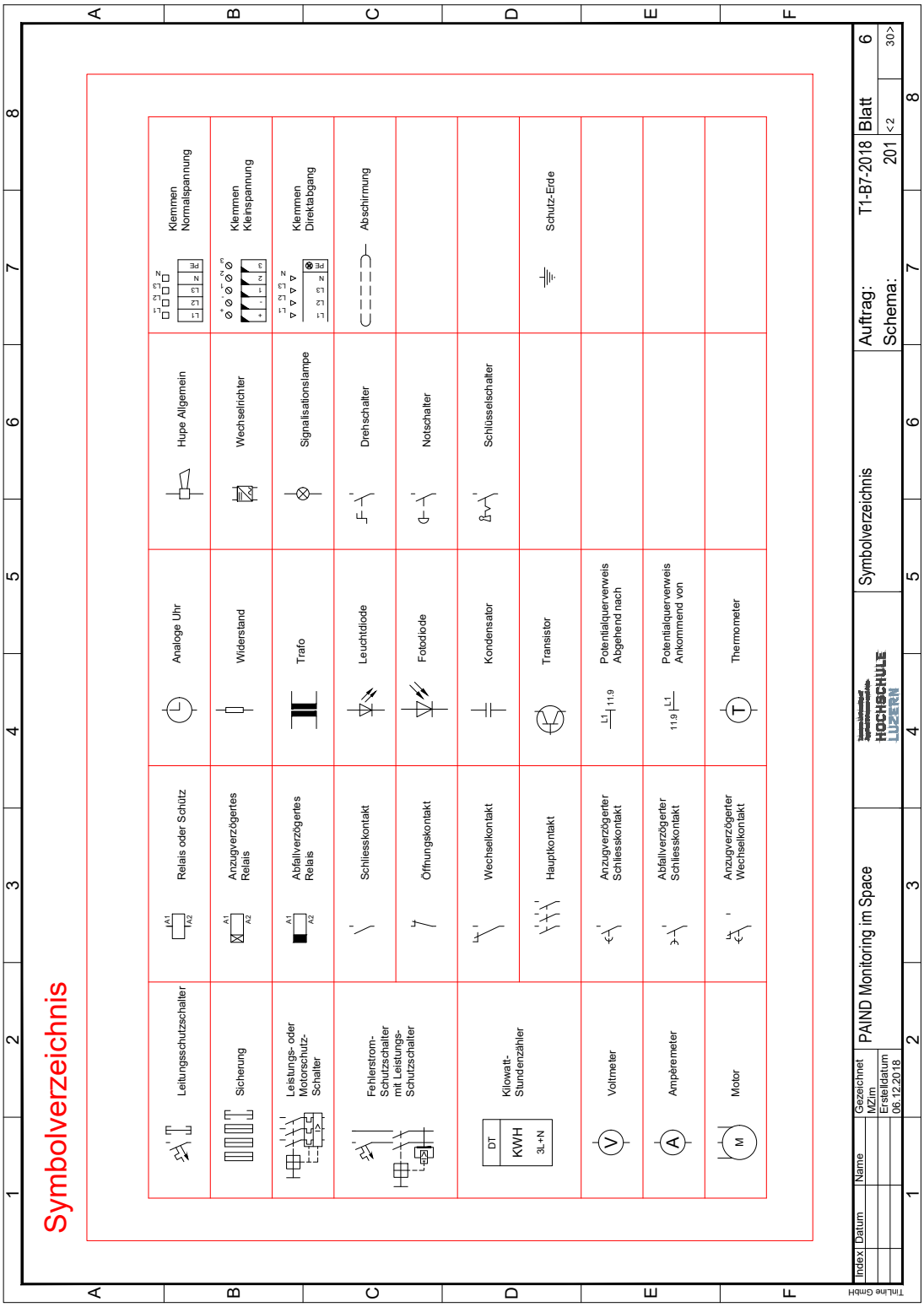
2. Middle part of chamber

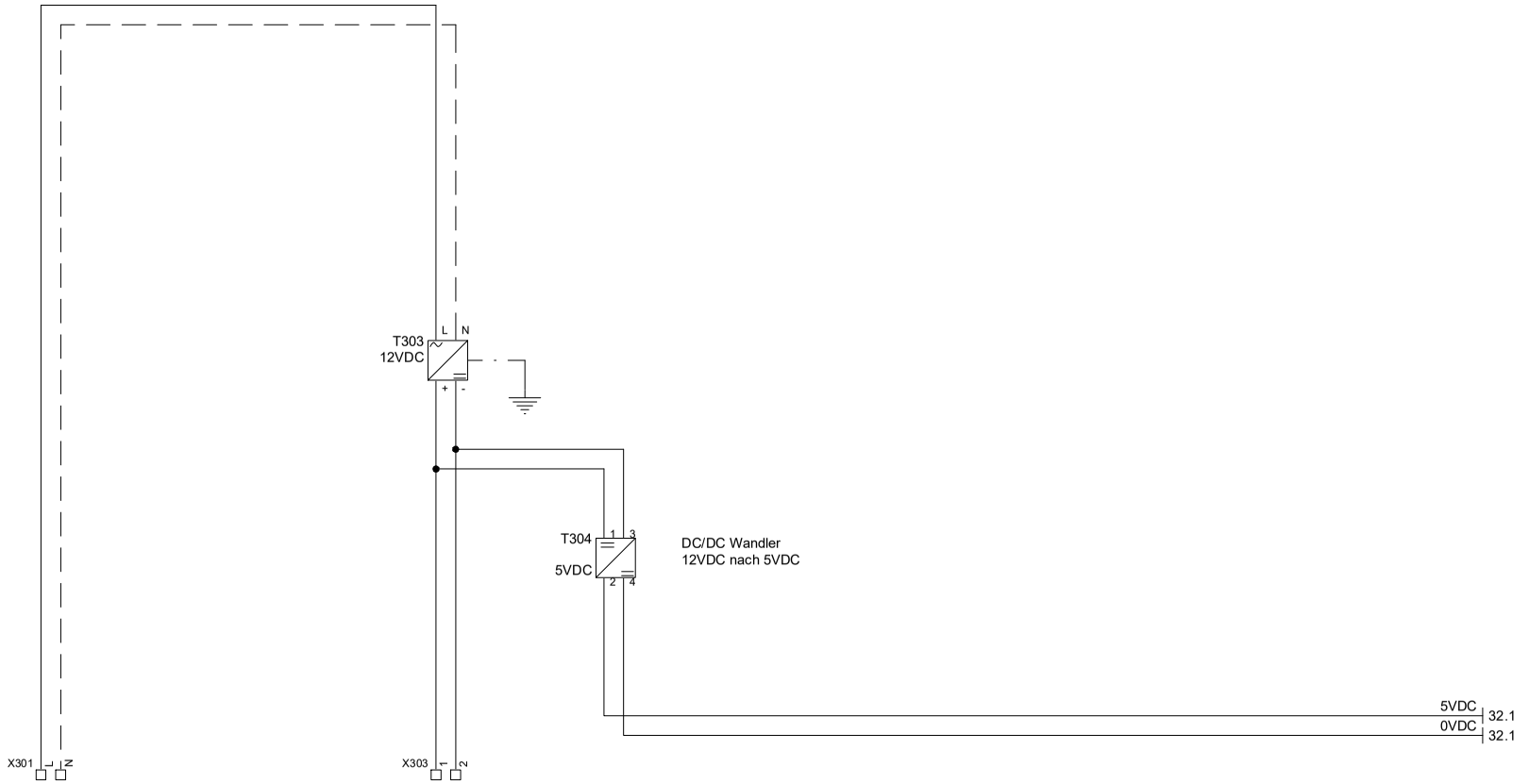


3. Bottom part of chamber



B.3 Sensors circuit





2.5mm2 — X301 — L, N, PE — Einspeisung — TT 3x1.5mm2

2.5mm2 — X303 — 1, 2 — 12VDC — TT 2x1.5mm2

Index	Datum	Name	Gezeichnet
			MZim
			Erstelldatum
			06.12.2018

PAIND Monitoring im Space

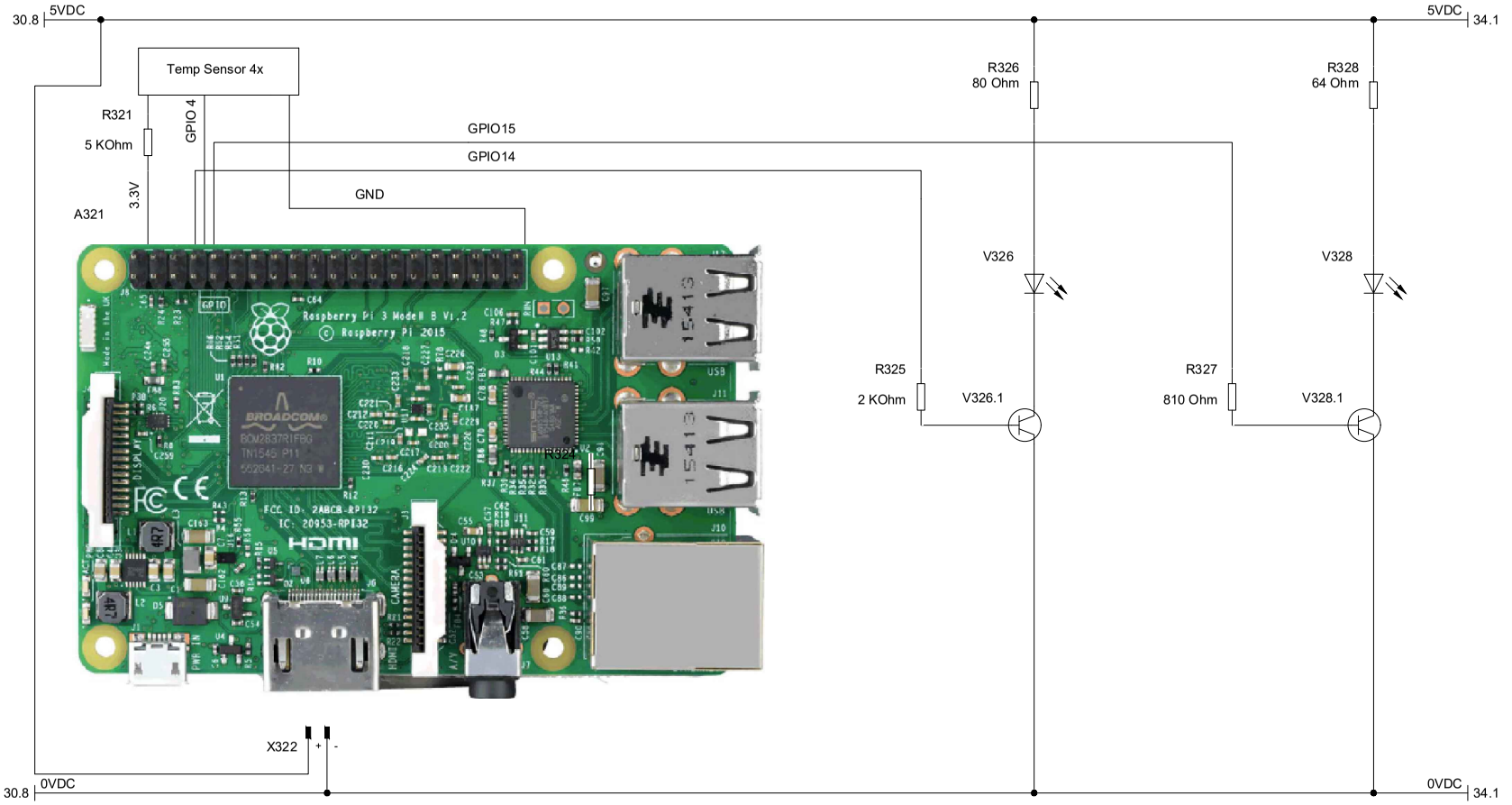
HOCHSCHULE LUZERN

Einspeisung

Auftrag: T1-B7-2018 | Blatt 30

Schema: 201 | < 6 | 32 >

TinLine GmbH



Index	Datum	Name	Gezeichnet
			MZim
			Erstelldatum
			06.12.2018

PAIND Monitoring im Space

HOCHSCHULE LUZERN

Rasperry Pi 3

Auftrag: T1-B7-2018

Schema: 201

Blatt 32

< 30 | 33 >

TinLine GmbH

Raspberry Pi B+ J8 Header

Pin#	NAME		NAME	Pin#
01	3.3v DC Power		DC Power 5v	02
03	GPIO02 (SDA1 , I2C)		DC Power 5v	04
05	GPIO03 (SCL1 , I2C)		Ground	06
07	GPIO04 (GPIO_GCLK)		(TXD0) GPIO14	08
09	Ground		(RXD0) GPIO15	10
11	GPIO17 (GPIO_GEN0)		(GPIO_GEN1) GPIO18	12
13	GPIO27 (GPIO_GEN2)		Ground	14
15	GPIO22 (GPIO_GEN3)		(GPIO_GEN4) GPIO23	16
17	3.3v DC Power		(GPIO_GEN5) GPIO24	18
19	GPIO10 (SPI_MOSI)		Ground	20
21	GPIO09 (SPI_MISO)		(GPIO_GEN6) GPIO25	22
23	GPIO11 (SPI_CLK)		(SPI_CE0_N) GPIO08	24
25	Ground		(SPI_CE1_N) GPIO07	26
27	ID_SD (I2C ID EEPROM)		(I2C ID EEPROM) ID_SC	28
29	GPIO05		Ground	30
31	GPIO06		GPIO12	32
33	GPIO13		Ground	34
35	GPIO19		GPIO16	36
37	GPIO26		GPIO20	38
39	Ground		GPIO21	40

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16/07/2014

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Index	Datum	Name	Gezeichnet
			MZim
			Erstelldatum
			06.12.2018

PAIND Monitoring im Space

HOCHSCHULE LUZERN

Pinbelegung Rasperry Pi 3

Auftrag: T1-B7-2018

Schema: 201

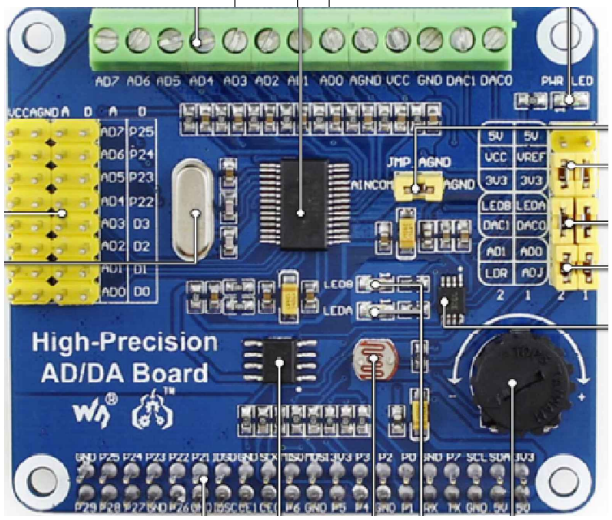
Blatt 33

< 32 | 34 >

32.8 5VDC

5VDC

A341



P344 1 O2 2

P345 1 pH 2

Fotodiode

5VDC

R346

1 KOhm

V346

C346

0.1uF

R347

0-90 KOhm

0VDC

32.8 0VDC

0VDC

AD Wandler

Index	Datum	Name	Gezeichnet
			MZim
			Erstelldatum
			06.12.2018

PAIND Monitoring im Space

HOCHSCHULE LUZERN

AD Wandler

Auftrag: T1-B7-2018 Blatt 34

Schema: 201 < 33 >

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