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**Experimental setup to measure the rate of oxygen
production from cyanobacteria**

Bachelor's Thesis (12 ECTS)

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Experimental setup to measure the rate of oxygen production from cyanobacteria

Abstract

This thesis aims to design and build an experimental setup that includes a novel oxygen sensor developed at Tartu Observatory, algae, specifically *Synechococcus*, for photosynthesis. This work is motivated by the need to test the oxygen sensor for future implementation in the International Space Station as well as allowing for future calibration of the sensor. The developed experimental setup incorporates control mechanisms to support the growth of *Synechococcus* cyanobacteria and monitor the oxygen production. The implementation and methodology of devising the required setup is discussed, highlighted key challenges and discovered solutions towards a stable experiment. The results obtained validate the functionality of the oxygen sensor over a large time period of continuous monitoring, verifying its usability in the experimental setup and laying the foundations to the eventual goal of utilizing it for future calibration of the oxygen sensor.

Keywords Oxygen Sensing, Photosynthesis, Automation, Cyanobacteria

CERCS: T320 Space technology, T490 Biotechnology, T110 Instrumentation Technology

Institute name: Tartu Observatory

Research group: Space Technology

Katse seadistus tsüanobakterite hapnikutootmise määra mõõtmiseks

Lühikokkuvõte

Selle lõputöö eesmärk on disainida ja ehitada eksperimentaalne süsteem, mis kasutab Tartu Observatooriumi välja töötavat uudset hapnikuandurit ning fotosünteesiks vetikaid, täpsemalt *Synechococcus*. Töö motivatsioon on vajadus testida hapnikuandurit tulevaseks kasutamiseks Rahvusvahelises Kosmosejaamas ning luua süsteem mida saaks kasutada anduri edasiseks kalibreerimiseks. Välja töötatud eksperimentaalne süsteem sisaldab kontrollmehhanisme, mis toetavad *Synechococcus* tsüanobakterite kasvu ja võimaldavad jälgida nende hapnikutootmist. Töös käsitletakse süsteemi loomise metoodikat ja teostust, tuuakse esile peamised väljakutsed ning kirjeldatakse leitud lahendusi stabiilse katse läbiviimiseks. Saadavaid tulemusi kasutatakse hapnikusensori töökindluse pikaajalise pideva

monitooringu jaoks tõestamaks selle sobivust eksperimentaalses süsteemis kasutamiseks ning rajades aluse sensori edasisele kalibreerimisele.

Võtmesõnad: Hapnikuandurid, Fotosüntees, Automatiseerimine, Tsüanobakterid

CERCS: T320 Kosmosetehnoloogia, T490 Biotehnoloogia, T110
Instrumentatsioonitehnoloogia

Instituudi nimi: Tartu Observatoorium

Uurimisrühm: Kosmosetehnoloogia

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TERMS, ABBREVIATIONS AND NOTATIONS

CAD - Computer Aided Design

GPIO - General-Purpose Input/Output

IDE - Integrated Development Environment

LED - Light Emitting Diode

MHD - Magnetohydrodynamic

PLA - Polylactic acid

INTRODUCTION

Recently, Tartu Observatory has been working in collaboration with a Polish company, “Extremo Technologies”, on a project called “Space Volcanic Algae”, that will be implemented on board the International Space Station [1] with the launch planned on the 8th of June as part of the Ignis mission [2]. For this collaboration, Tartu Observatory needs to develop a reference setup for an oxygen sensor to learn how it behaves in real experiment conditions, to later interpret the data coming from the experiments on ISS [3]. While a new version of the sensor was developed for use in the ISS, for this reference measurement, we are using an earlier sensor, a luminescence-based optical oxygen sensor built by Dr. Pajusalu at the Massachusetts Institute of Technology(MIT) [4]. This sensor works by measuring the luminescence lifetime of luminescent dye, which changes based on the partial pressure of oxygen in the gas. The values of oxygen partial pressure can be related to the values of the luminescence lifetime, which can be done by calibrating the sensor. To do this, multiple experiments need to be conducted, so that the sensor can be tested under different circumstances, and from them, data needs to be recorded, which is the exact basis for this thesis.

The main problem is that at present, there is no designated experimental setup that can be used to perform a realistic reference measurement for testing, recording data from, validating, and calibrating the sensor, to understand how the sensor works in realistic conditions. Therefore, the key objectives for this project are to first design an experimental setup that includes cyanobacteria in a glass vessel for oxygen production via photosynthesis, a good aeration system, and lights for photosynthesis. In addition, the system must be automated to enable both remote monitoring of measurements and off-site control of environmental parameters such as light and aeration.

In this study, an optical oxygen sensor [4] is evaluated for future use in space-related biological research. The oxygen sensor is used to observe and quantify the oxygen level changes during cyanobacteria's photosynthesis in real time. In the scope of this work, the oxygen produced during cyanobacteria's photosynthesis is used as a biological reference, which involves measurement of oxygen produced by the cyanobacteria cultures, analysis of the experimental setup's reliability, and troubleshooting limitations.

1 LITERATURE REVIEW

1.1 Oxygen sensors

Oxygen sensors are devices that can be used to determine oxygen concentration in gases and liquids. Being able to measure oxygen levels in the environment accurately can be essential for various fields such as geology, microbiology, geobiology, chemistry, and material science [4]. In geology, determining oxygen concentration can help us understand the rate at which the appearance of minerals changes depending on it. Broader use of the sensors is that they can be used to quantify oxygen diffusion in systems and confirm the quality of preparation facilities like anaerobic chambers [4]. Additionally, measuring oxygen can be useful in astrobiology for experiments studying the metabolic activities of microorganisms in simulated environments. They can detect if microbes are consuming or producing oxygen in set conditions, helping to identify biosignature gases and the possibility of life on exoplanets with unusual atmospheric makeup, such as environments with a 100% hydrogen atmosphere [5].

1.1.1 Overview of oxygen sensor technologies

Oxygen concentration can be measured using different technologies. One of the most popular ways is to use a Clark-type Oxygen sensor, which is particularly useful in biological systems such as cell cultures. Clark sensors operate by incorporating a cathode, an anode, an electrolyte solution, and a gas-permeable membrane. They have the advantage of being low-cost and having a rapid response time. However, they also have some design-specific disadvantages. An example of this is that the local oxygen concentration may be affected by the consumption of dissolved oxygen at the working electrode. [6]

Being able to accurately measure oxygen in nanomolar stages can have some very important applications, however, there are limited sensors that can actually do that. One such sensor is an electrochemical STOX sensor, which can be used in cases of laboratory and in-situ studies, and can measure oxygen concentrations down to 10nM, however, they are very fragile and difficult to build. A good alternative to electrochemical sensors in this case is luminescence-based optical sensors. Such sensors work by detecting a change in luminescent lifetime or intensity, which begins with changes in oxygen levels. Unlike the previously mentioned Clark-type sensor, these sensors have the advantage that they do not consume the oxygen they are measuring. Furthermore, they can be made very small and are non-invasive, and work through an optical window. [7]

1.1.2 Working principle of optical sensor

In this experiment, an open-source luminescence-based optical sensor for measuring oxygen partial pressure, developed by Dr. Mihkel Pajusalu et al. was used[4](Figure 1). This sensor measures the oxygen concentration by detecting changes in the luminescence lifetime of a luminescent dye on a patch attached inside the vial above the photodiode used for recording the luminescence intensity as the sensor system modulates a Light Emitting Diode(LED) with a sinusoidal excitation waveform. This works because the luminescence lifetime of the dye depends on the partial pressure of oxygen in the environment. The sensor system regulates an LED with sinusoidal excitation and documents the emission waveform with a photodiode. This sensor measures the partial pressure of oxygen in the gas phase. The sensor can connect to WiFi and can send all the accumulated data over the network or through a USB cable.

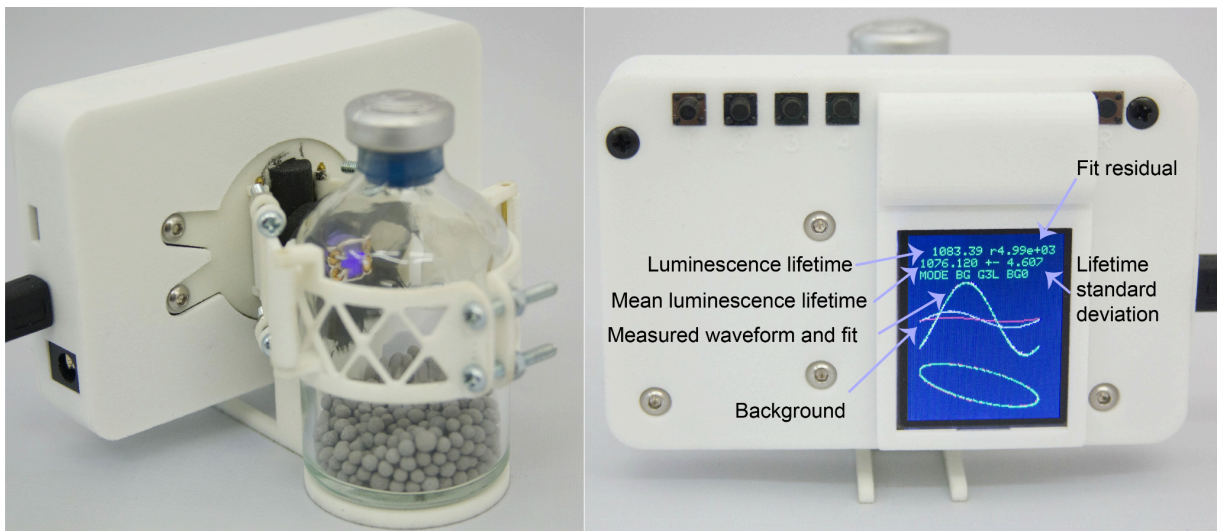


Figure 1: open-source luminescence-based optical sensor for measuring oxygen partial pressure[4]

To be able to fully understand the oxygen levels based on the readings from the sensor, it is first necessary to calibrate it, which can be done by first flushing the vial with high-purity nitrogen gas (containing less than 5 ppm oxygen) to eliminate residual oxygen, followed by a stabilization period to allow diffusion-driven equilibration with ambient oxygen levels. This is taken as the 0 point for oxygen concentration.

Then, to generate calibration points, known volumes of atmospheric gas can be slowly injected into the vial to introduce known quantities of oxygen. These additions showcase the effect of oxygen partial pressure on the sensor's luminescence lifetime measurements[4]. To be able to achieve this, first, an experimental setup needs to be built that can be used for testing the sensor, as shown in Figure 2.

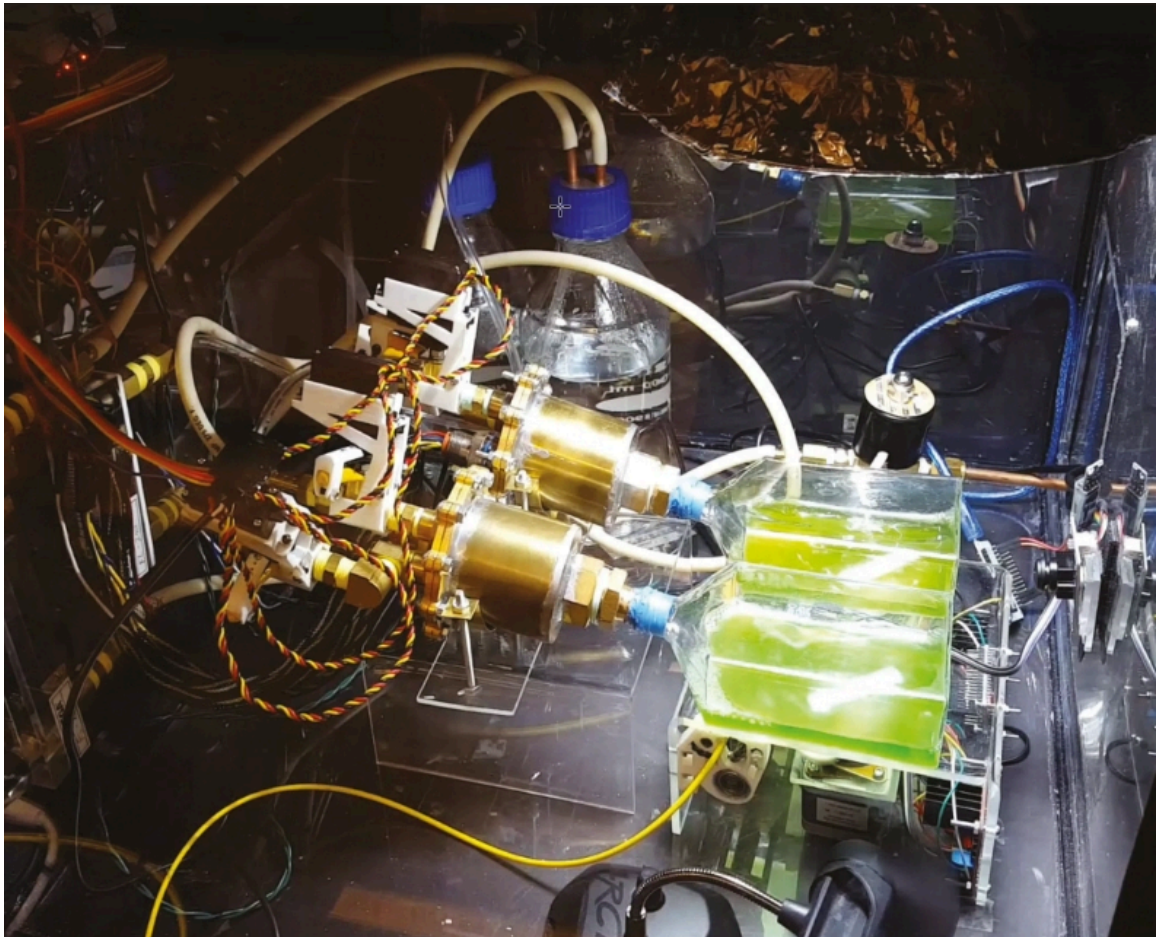


Figure 2: Original setup done by Dr. Pajusalu at the MIT

1.2 Photosynthetic oxygen production by cyanobacteria

Algae are a polyphyletic group, meaning they don't share their most recent common ancestor. However, in autotrophic eukaryotes like red algae, green algae, and plants, photosynthesis takes place in chloroplasts that are descended from ancient cyanobacteria through endosymbiosis [8]. Cyanobacteria are autotrophic, prokaryotic algae, they use pigments like chlorophyll, carotenoids, and phycobilins to turn sunlight into chemical energy using photosynthesis [9]. Cyanobacteria have internal membranes called thylakoids, where this process happens [10]. Cyanobacteria play a crucial role in oxygen production on Earth. Roughly one-half of all production happens in the ocean, and despite hundreds of species participating, *Synechococcus* and *Prochlorococcus* are responsible for around 25% of all oceanic production [11].

Because of their simple growth requirements and rapid doubling rate, *Synechococcus* is one of the most popular cyanobacteria for research purposes [12]. For example, in space research, cyanobacterium *Synechococcus* 2973 is planned to be engineered to create food such as beta-carotene, nutraceuticals, and biopolymers from human waste produced in space [13]. The

idea of using algal photobioreactors to create bioregenerative life support systems has been around for a long time, and since in the future, space exploration will take the astronauts further away from Earth, beyond the Moon, for longer times than 6 months, with high difficulty in supplying resources from outside, self-sufficient space stations are becoming a necessity for the evolution of space research [14].

1.3 Sensor Applications in Space and Closed Systems

The oxygen sensor used in this work [4] has been used in a study about the viability of life in H₂-dominated exoplanet atmospheres [15]. The study aimed to test whether single-celled microorganisms like *Escherichia coli* and yeast could survive and grow in a 100% H₂ atmosphere, which is relevant to the search for life on exoplanets with expansive, low-density H₂ atmospheres. The sensor was used to make sure the experimental environment was truly H₂-dominated and to accurately measure any evidence of oxygen being present, which, even at the smallest amounts, could affect the growth and metabolism of microorganisms [16].

Another application of this sensor was studying the limits of active flight in a low-density atmosphere [17]. The setup included a cell culture bottle, to which instant *Drosophila melanogaster* medium was placed along with deionized water and active dry yeast. Using the oxygen sensor, by proxy, the concentrations of other gases could be determined, and consequently, the atmospheric density within the experimental container. This was crucial to correlate the observed fly behaviours with specific atmospheric conditions. Furthermore, by carefully tracking O₂ concentrations, researchers could ensure that any observed limitations in flight were primarily due to atmospheric density rather than a lack of oxygen for metabolic processes

1.4 System integration and automation in biosensing

Automating a biosignature setup can be an invaluable addition to an experiment. Firstly, it allows less human effort to be used on manual tasks such as regulating different environmental factors, in this experiment, the light and the pump. It also allows one to perform sensing activities remotely. Furthermore, an automated system can also easily be used by people who do not have experience with certain aspects of the experiment. For this experiment, it means that this setup can be used even by someone who does not understand electronics or the coding aspect of it, but still needs the data from sensing.

1.4.1 Microcontrollers and single-board computers

Microcontrollers and single-board computers play a crucial role in building and automating such systems. Many biosensors have an interface compatible with them to convert analog signals into digital data for further analysis.[18] One of the most commonly used, cost-efficient, and practical single-board computers for automation purposes is the Raspberry Pi. Raspberry Pi is a United Kingdom-manufactured series of small, single-board computers capable of high-performance-demanding tasks. Examples of its use can be found in physical computing(controlling hardware with Python), building games in Scratch, and setting up camera projects. It is easy to use and good for beginners.[19]

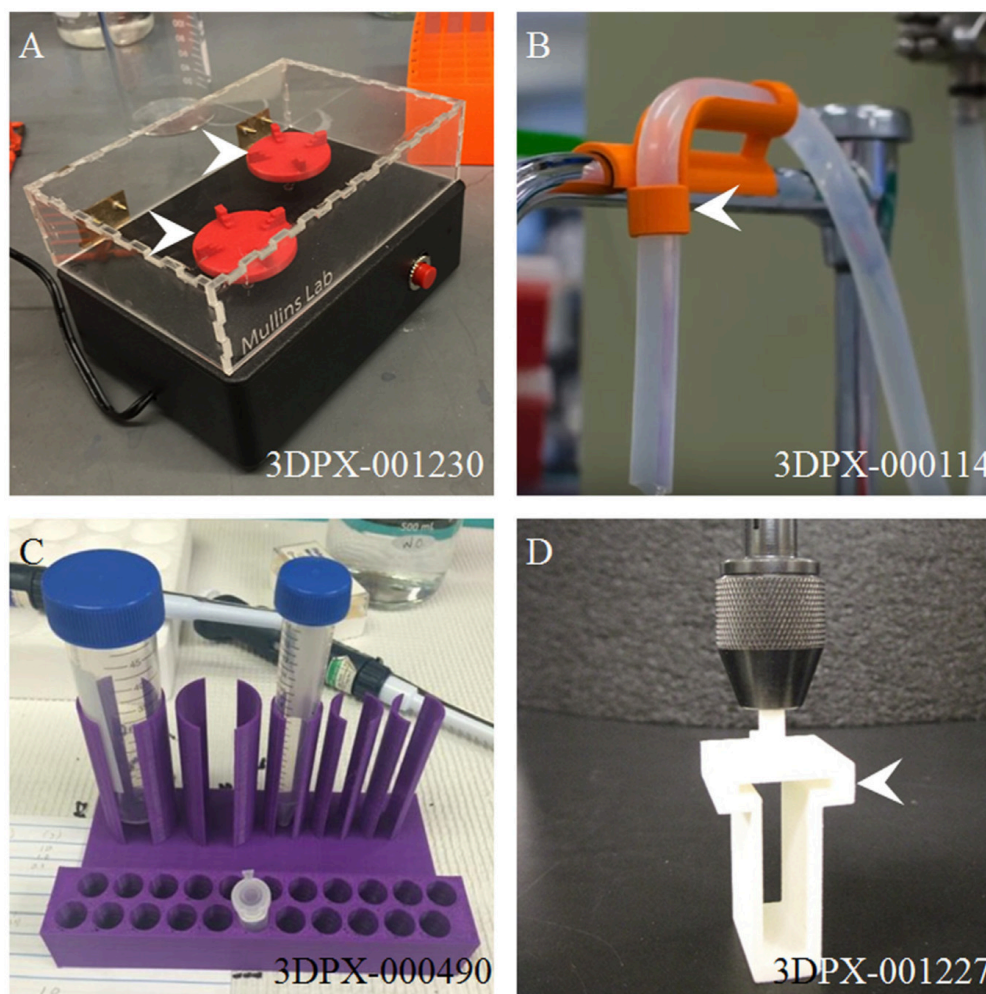
Throughout the past decade, there have been several papers released describing how to implement a Raspberry Pi to automate your home and turn it into a smart home.[20], [21],[22]. Raspberry Pi has found its place in biological applications as well. Its cost effectiveness, data acquisition style, and programmability has made it a valuable tool for field researchers, for remotely observing wildlife. An example of that is that it was used to build a nest camera system to record the behaviour of Acorn Woodpeckers (*Melanerpes formicivorus*) in artificial tree cavity nests. Along with the Raspberry Pi, they used a Pi NoIR infrared camera, a wireless adapter to transmit video over the internet, and Deka rechargeable gel batteries for power. The system was programmed so it would motion-sense and record only during daylight hours, after which it would automatically upload the videos over the wireless server to the cloud.[23]

To this project, a more relevant comparison would be the automation of a laboratory system. The rise in open-source, low-priced electronics has revolutionized experimental setups. It has been quite useful for automatic flow control using pumps for microfluidic studies. This is especially necessary now that there is an increase in research projects involving lab-on-a-chip[24] devices, and their correct function depends on accurate fluid manipulation for filtration, mixing, and separation of components, which is being done by multiple micropumps(small pumps that can move liquids nano-to microliters) and pneumatically actuated microvalves, which are small valves that can be closed and opened with air pressure or vacuum. An example of such a setup is by R. M. Cardoso et al., where automation of the system is done on an Arduino, which they used along with relays to control multiple magnetohydrodynamic(MHD) pumps and pneumatic valves[25].

For the scope of this thesis, a Raspberry Pi 5 was used, as it provides sufficient computational power for running real-time data logging and control loops. Its GPIO (General Purpose

Input/Output) Pins allow direct control of hardware components like relays, LEDs, sensors, and pumps, which are crucial for regulating environmental variables like light intensity and aeration. It has multiple USB ports, which are needed for the connection with the sensor and the magnetic stirrer. Moreover, its networking capabilities, both Wi-Fi and Gigabit Ethernet, allow for remote monitoring and control of the system, which is a critical functionality for experiments that run over long periods and require off-site access [26].

1.5 3D printing in laboratory system design



3D printing, also known as Fused Deposition Modeling (FDM), is one of multiple additive manufacturing methods popular for both large-scale production and personal use. Unlike the traditional way of moulding the material into the desired shape, 3D printing creates objects through a layer-by-layer deposition of material directly guided by a computer-aided design(CAD) model[27]. The implementation of 3D printing into scientific research has revolutionized the development of tools used for research by allowing low-cost, easily customizable, precise, and fast prototyping solutions. Some examples of this are components such as device casings, mounting brackets, connectors, holders, etc (Figure 3)[28]. Without

the 3D printing possibilities, creating these components would be less accessible and slow down the speed of research. *Figure 3: Example of parts produced using 3D printing technology for laboratory [28]*

2 THE AIMS OF THE THESIS

The aims of the thesis should be written out point by point.

- Develop a physical setup that measures the changes in oxygen levels as a result of cyanobacterial photosynthesis, using an oxygen sensor.
- Automate the experimental setup by programming and wiring the control aspects of it, an LED light, and an air pump for the environmental control.
- Grow cyanobacterium *Synechococcus* under a controlled laboratory environment using a custom-built system that includes light exposure and aeration control.
- Validate the oxygen sensor operation by relating the sensor output to expected outcomes of known biological processes, such as changes in light/dark cycles, on/off cycles of the pump, etc.

3 EXPERIMENTAL PART

3.1 MATERIALS AND METHODS

3.1.1 Growing cyanobacteria

In this experiment, cyanobacteria *Synechococcus* [29], [30] were used. Obtaining biological cultures or materials like media from a specialized laboratory supply company can be problematic, due to the high price and long delivery times, especially for small quantities. For this project, alternative sources were used. *Synechococcus* was procured from two different sources. One from an online aquarium store called “Ocean Store” [29], and the other from a Dutch company, “Phydrotec” [31], through an online marketplace “Etsy”, along with F2 media and sea salt. Different strain cultures were pre-grown in the media on an orbital shaker with cycled plant growth light, as can be seen in Figure 4. Orbital shakers are usually considered an ideal mechanism for mixing the liquid culture inside the vial because of their gentle, uniform motion; however, in this work for practicality, a magnetic stirrer was used instead. The vial with *Synechococcus* had a stirrer magnet inside and was put on top of a magnetic stirrer for even mixing of the medium, which prevents any cells from settling at the bottom of the vial and helps each cell get an equal amount of exposure to light and nutrients, which in turn helps with uniform growth and photosynthesis, as shown in Figures 5, 6, and 7. In the experiment, marine *Synechococcus* was used, for which a saline environment is necessary. To replicate natural seawater conditions, sea salt was added to the F/2 media with a concentration of 35g/l. During this thesis, the exact strain of the *Synechococcus* used is unknown; however, since the specific strain does not affect the overall experiment setup it is outside the scope of the thesis. The experimental setup is being done at the Tartu Observatory laboratory, where the ambient temperature is regulated to room temperature, and aeration was provided with an aquarium pump. Culture was kept in a glass vial with a stopper to prevent any unwanted airflow, however, needles were inserted to let the gas in from the pump and let out anything extra air. Furthermore, filters were attached to needles since the final goal is for the whole setup to be sterile and prevent any unwanted bacteria from entering the open space; however, we did not have access to an autoclave, therefore, sterility could not be ensured. This was not a problem at this stage of the experiment, since the main idea was to see the photosynthesis happen and the fact that *Synechococcus* is commonly used in aquarium systems, where it manages to grow due to its advantage over other microorganisms, therefore, its ability to outcompete contaminant species makes it very unlikely that some external organisms would establish themselves. Despite this, the setup was designed to be as accurate

to the final objective as possible. The culture was provided with a light cycle of 12:12 hours using 3 plant growth LED lights.

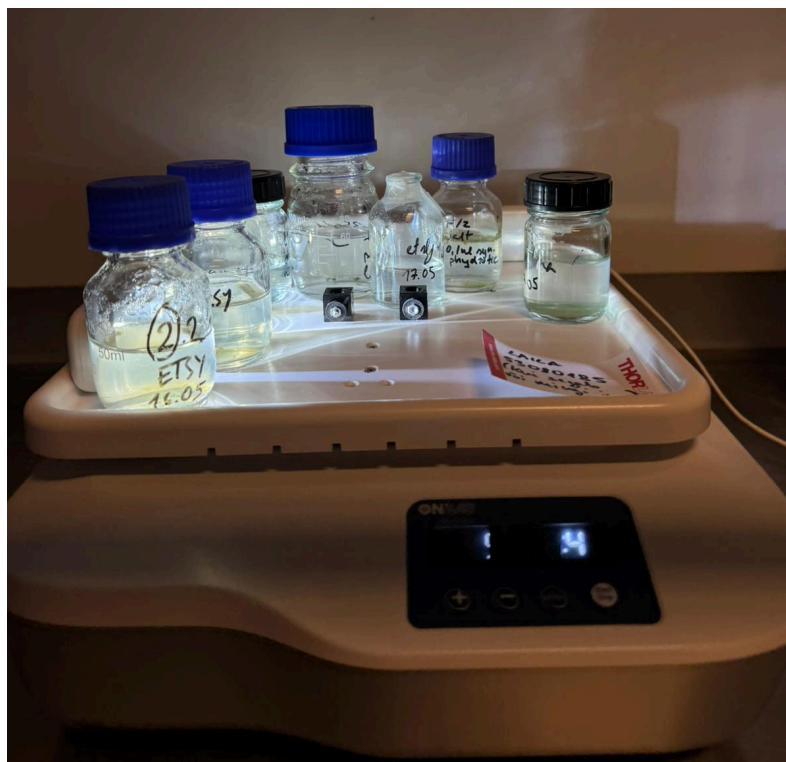


Figure 4: Multiple cyanobacteria cultures growing on an orbital shaker

3.1.2 Experimental setup

To observe the photosynthetic oxygen production, a custom setup was assembled, using a gas-flow system consisting of an air pump connected to the culture vial with silicone tubing, check valves to prevent the air backflow, and a humidifier, which is a sealed bottle with distilled water and tubing going in and out. The inflow tube has a needle attached that reaches into the water to confirm the gas input with bubbles created. The gas is being let inside and outside the vial with tubing attached to needles as well. The vial with culture is put inside the holder attached to the oxygen sensor so that the luminescent dye patch is positioned correctly, and the vial, along with the oxygen sensor, is set on the magnetic stirrer. For photosynthesis of the cyanobacteria, an LED light was used, to which the voltage supply, along with the pump, was being controlled by a relay board, which in turn was being managed by a Python code on the Raspberry Pi. To make the system more sturdy and eliminate some physical errors, and to cover the electronics, 3D printed models were implemented.

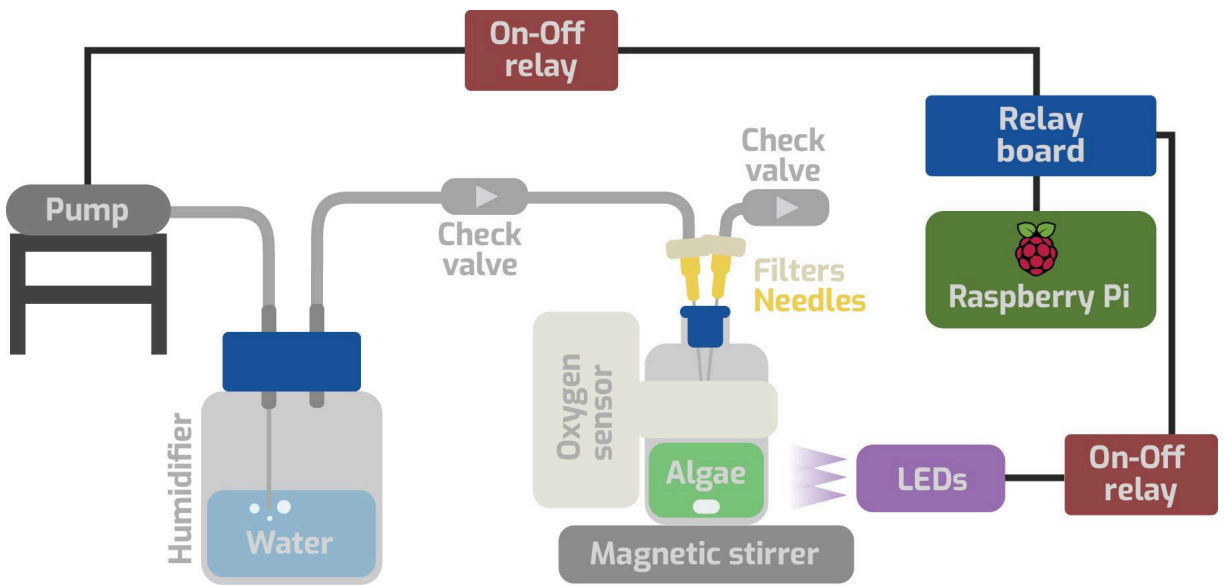


Figure 5: Scheme of the full setup

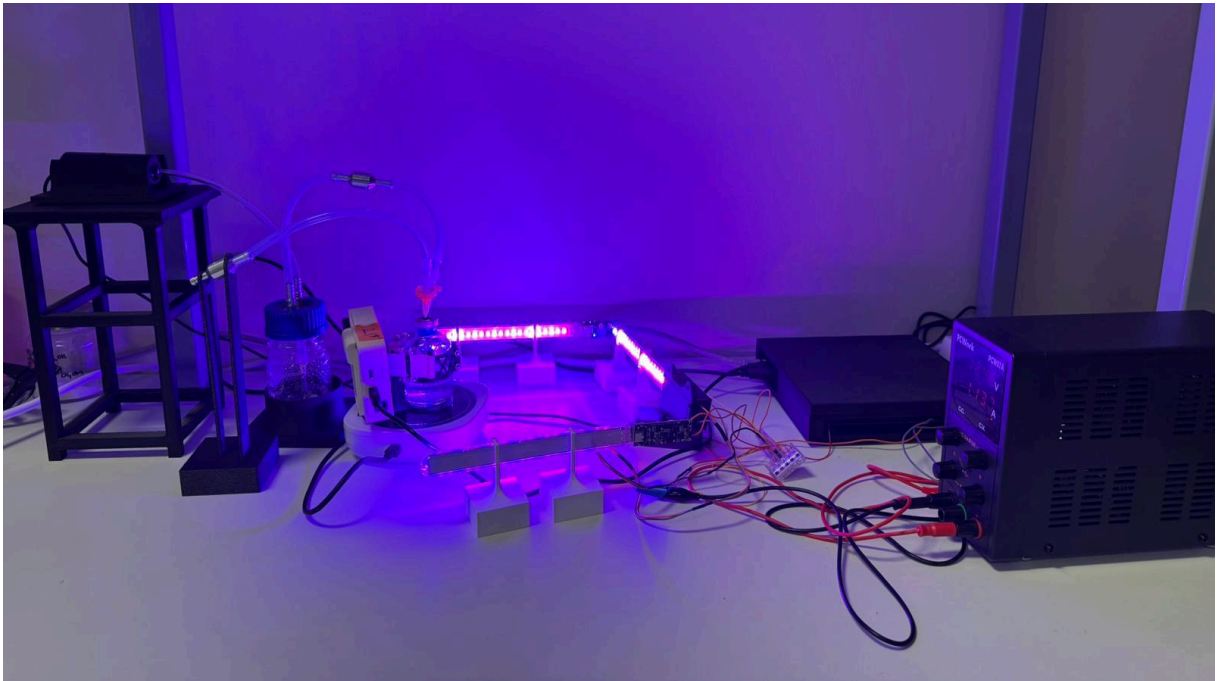


Figure 6: Full setup light ON cycle

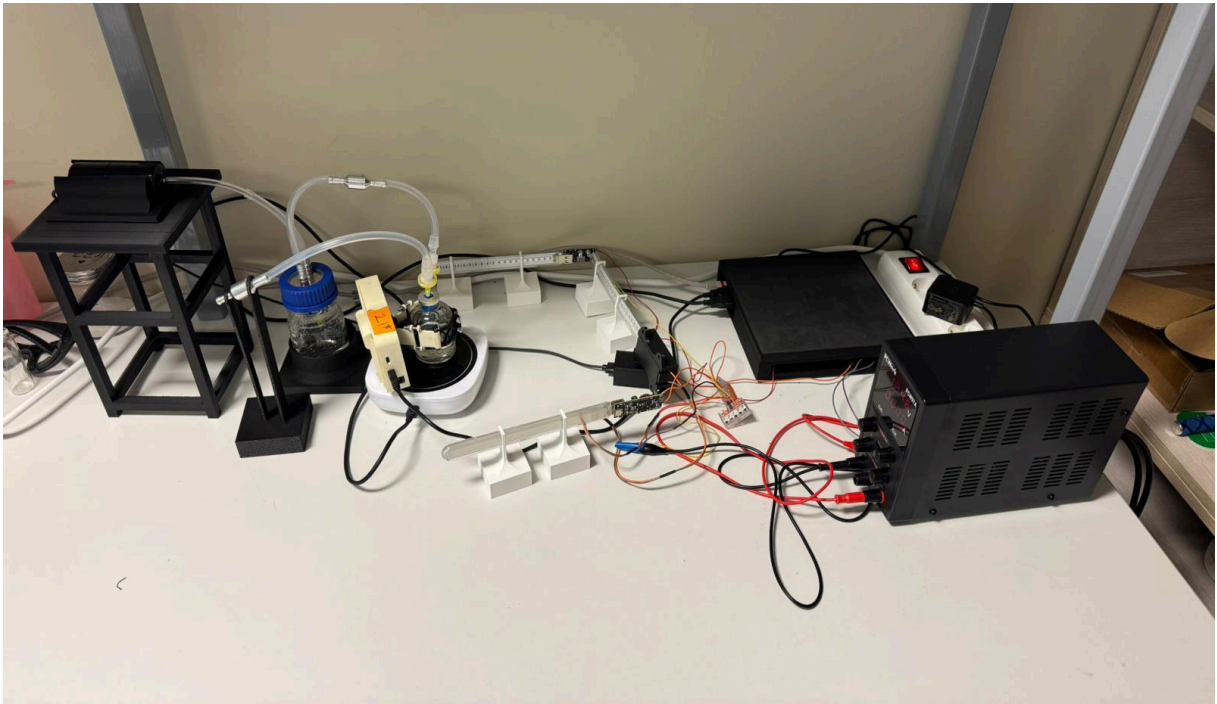


Figure 7: Full setup light OFF cycle

3.1.3 Electronics and automation

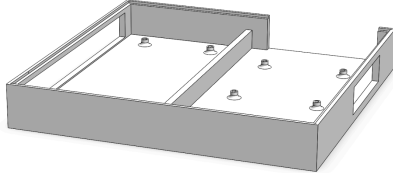
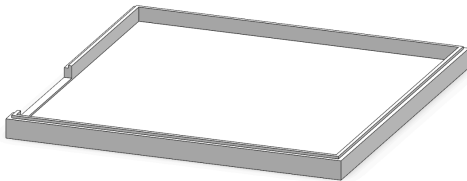
A Raspberry Pi was used as the main controller for data logging and system automation. Raspberry Pi was powered through a power multiplier, which was connected to a standard mains outlet. The LED light and the air pump were powered through a laboratory power supply PCW07A [32], by PCWork, which itself was also connected to a standard mains outlet, and to control the voltage supply to these components through a Python script, an 8-channel 5V relay module [33] was used. To connect the air pump and the lights to the power supply, first, each of them was connected to a DC/DC step-down power module (12V/24V to USB QC3.0) [34], which regulated the voltage and enabled a safe and reliable power delivery to the components. For practical wiring, all the positive leads of the light system(all three LED lights) were inserted into a WAGO cable connector [35] along with one jumper wire [36]. The other end of the wire was inserted into a Normally Open(NO) terminal of a single relay channel. From the Common(C) terminal, another jumper wire was connected to a 12V power input via the banana connector of the PCW07A. All the negative leads of the lights and the pump were again inserted into another WAGO connector with the same mechanism of an extra jumper wire, the other end of which was directly connected to the negative banana connector of the power supply. The positive lead from the pump was inserted into a Normally Open(NO) terminal of a relay channel, and from the Common(C) terminal, a new jumper wire was connected to the 12V power input, also with the banana connector of

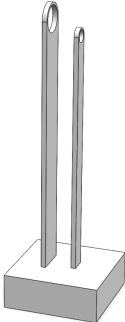
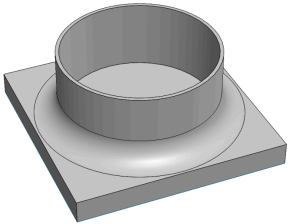
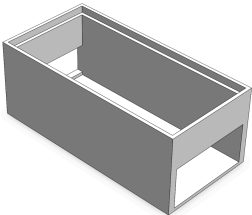
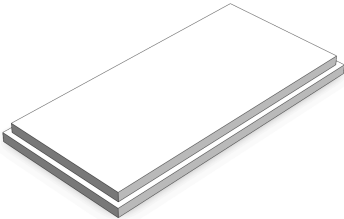
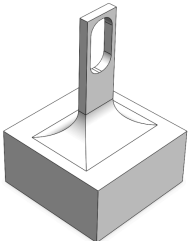
the PCW07A. The oxygen sensor and the magnetic stirrer were connected to the Raspberry Pi with micro USB cables.

The Python script for receiving and logging the data from the oxygen sensor was provided by the supervisors and later altered to record the measurements using not only the Unix timestamp, but also the actual date and time, in addition to gracefully handling small physical errors, such as the sensor disconnecting for a few seconds due to an unreliable microUSB cable. For the automation of the light and pump cycles, another Python script was used. The logic for both cycles was a simple time-dependent one. The lights were coded to be on from 9:00 to 21:00 and off from 21:00 to 9:00, which completes its 12:12 cycle required for *Synechococcus* photosynthesis. The pump was coded to be on for 2 minutes and off for 30 minutes. This allows for the vial to be replenished with the necessary amount of carbon dioxide for photosynthesis in 2 minutes, and the 30 minutes off lets the oxygen build up inside the vial enough for the sensor to be able to note the difference.

3.1.4 3D printing new parts

In this project, 3D printing was utilized to manufacture some custom parts required for the assembly and operation of the experimental setup. Components were designed using SolidWorks [37] and printed using Bambu Labs P1S 3D printer [38] at Tartu Observatory, using Polylactic acid(PLA) as the primary filament type [39].

No.	Model	Description
1		Casing for the Raspberry Pi and the relay channels. This is so that there are no exposed electronics.
2		Lid for the casing.

3		<p>A support structure for the check valve to ensure stable positioning and prevent unsupported suspension</p>
4		<p>A support stand for the humidifier, to enhance stability and reduce the risks of accidental movement and tipping.</p>
5		<p>A protective casing for the USB converters to shield the electronics</p>
6		<p>Lid for the casing of the USB converter.</p>
7		<p>Supporting, a heightened stand for the LED light for optimal positioning.</p>

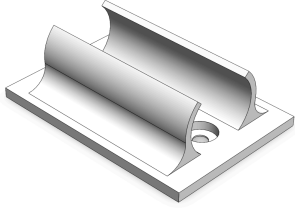
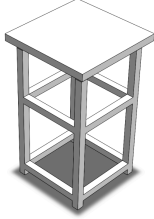
8		Casing for the pump to ensure its stability.
9		A heightened stand for the pump, so we can use the gravity affecting the ingoing gas towards the check valve to our advantage .

Table 1: Custom-manufactured parts using 3D printing

3.1.5 Implementation Aspects

The first few experiments were done with an original setup, shown in Figure 8, that included an already available aquarium pump and one LED light. We used both batches of *Synechococcus* separately.

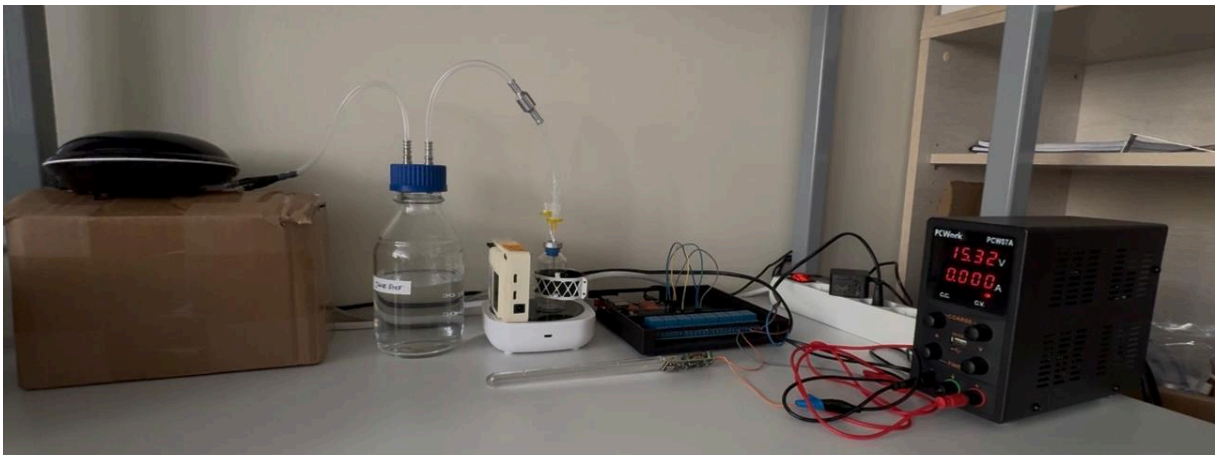


Figure 8: Original full setup using a different aquarium pump and one LED light

The second setup was built using a 50W LED grow light [40] and a different, stronger aquarium pump shown in Figure 10. To test and compare the strengths of the pumps, we conducted a small experiment in which we directed the airflow from each pump, after it had passed through the entire tubing setup, into a vial or a beaker with water, to observe whether or not it could produce bubbles (Figure 11). With the first pump, there was no bubbling. With

the second pump, there was strong, continuous bubbling, which suggested it was good enough for this experimental setup.

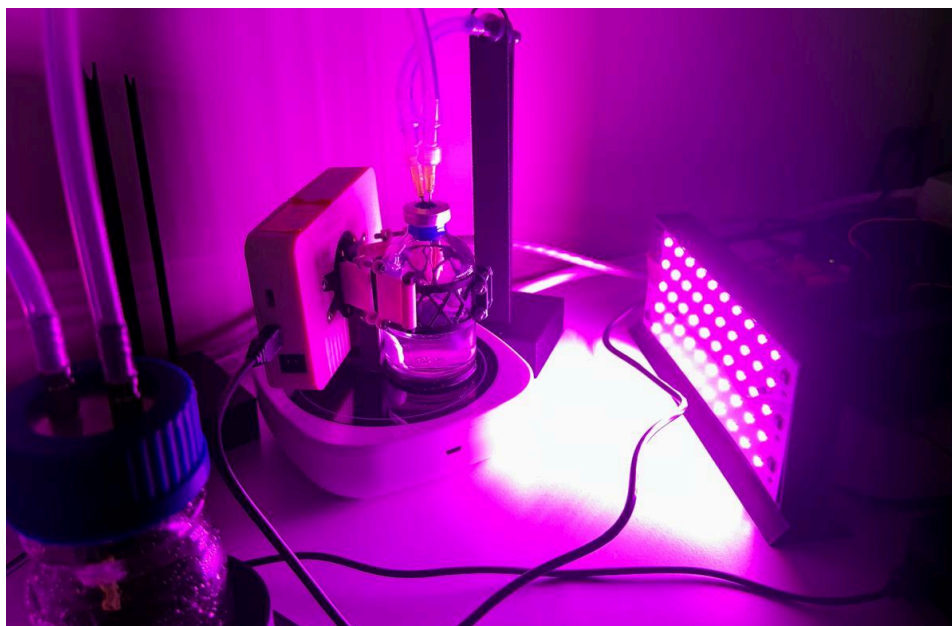


Figure 9: 50W LED light



Figure 10: The pump used in the final experimental setup

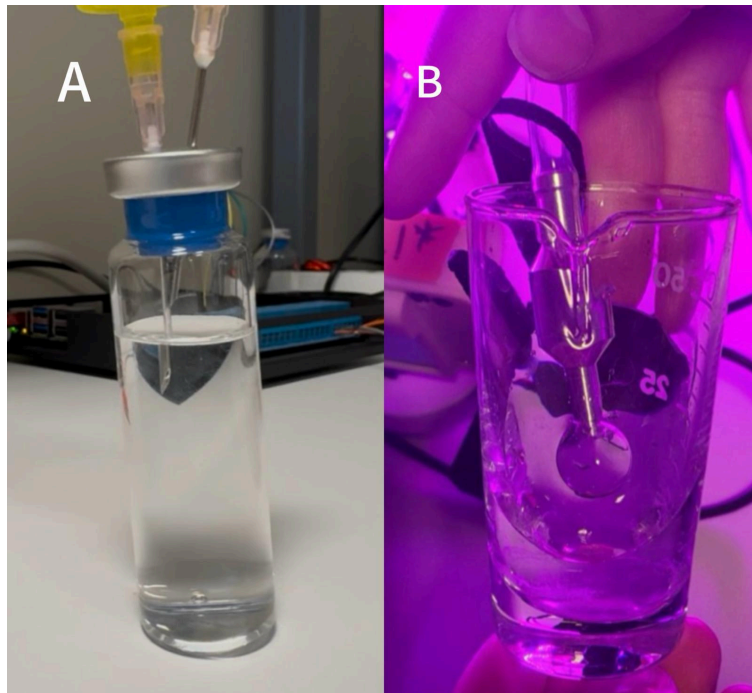


Figure 11: Comparison of the bubbling test between the first weaker pump(A) and the second stronger pump(B)

After this, we decided to try a different light approach and tested the setup using the light source[41] shown in Figure 12, which finally showed the expected results of photosynthesis, which can be seen in Figure 13. This experiment was run for 3 days. Since this implementation was just to test the setup over a few days and not for long-term use, there was no effort made to automate it, and instead, the light cycle was regulated manually.



Figure 12: Testing the setup with KuupKulgur light

3.2 RESULTS, ANALYSIS, AND DISCUSSIONS

The first coherent results were gathered when running the setup using the big light source (Figure 12). Right after starting the experiment, there was a clear trend of luminescence lifetime going down, which implies oxygen production, followed by a small rise during the dark - oxygen levels going down.

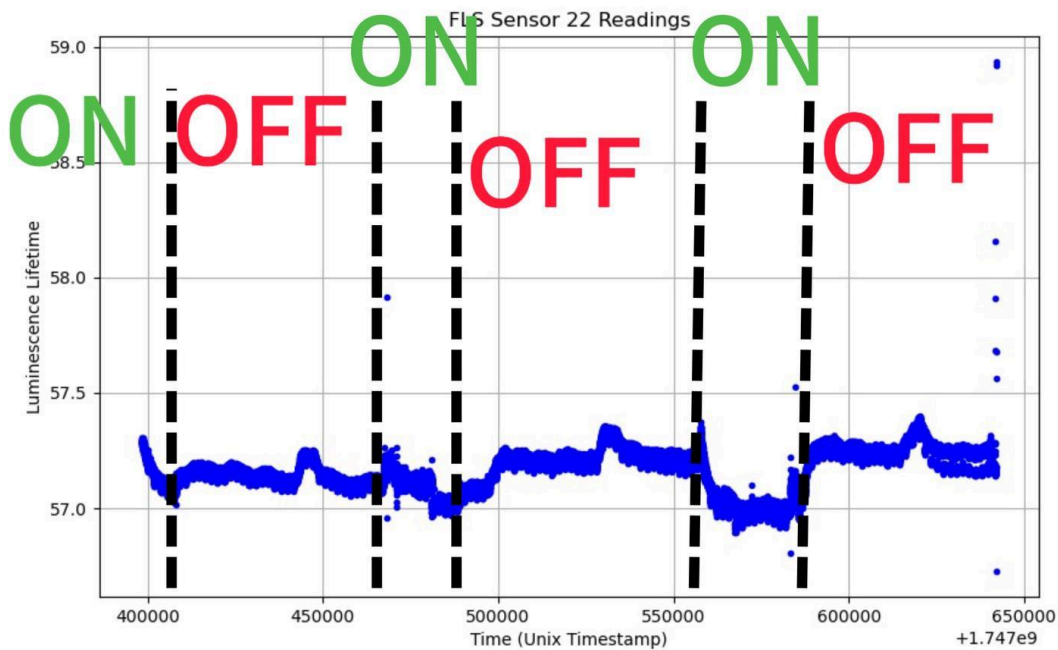


Figure 13: Graph generated from the measurements gathered during the experiment using stronger light[41] and the stronger pump, showcasing ON and OFF parts of the light cycle

After running the experiment using the most optimal setup(Figures 6 and 7), the oxygen sensor readings showed an expected result of decreasing luminescence lifetime, signifying oxygen production and buildup during the light ON cycle, followed by an increasing trend of luminescence lifetime, signifying lowering oxygen levels, during the light OFF cycle, which can be explained by the *Synechococcus* consuming oxygen in dark and continuing atmospheric flushes of the vial with the gas pump every 30 minutes. Furthermore, at each pump ON cycle, there is a small drop in the luminescence lifetime, which rises again as the pump turns off. This is thought to be the result of rising oxygen partial pressure as the pump injects the atmospheric gas in, despite the actual oxygen concentration not rising but instead lowering due to gas exchange. Despite seeing the expected results, there is still some noise that can not yet be explained

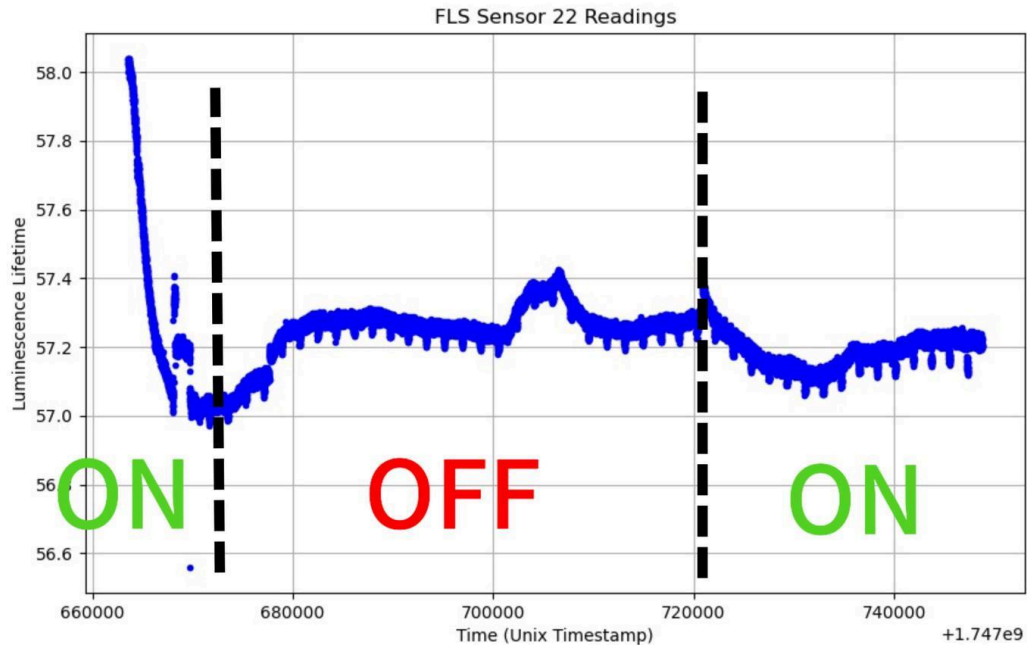


Figure 14: A graph illustrating the sensor readings with the final setup

3.2.1 Limitations

Throughout the experiment period, with different setups, several limitations and bottlenecks were discovered. During the first experiment in the sensor measurements, there were no indicators of oxygen production, which led us to understand some errors in our approach. First, the pump was too weak to provide any real push against the check valves and the filters, which means there was almost no CO₂ provided to the cyanobacteria after inserting it inside the vial, which killed it. This hypothesis was strengthened by comparing the results of the bubbling experiment between the weaker pump and the stronger pump. Furthermore, the light was not strong enough to induce photosynthesis in the cyanobacteria.

After trying the new setup with the new components, the experiment still did not seem to be producing any viable results (Figure 15). All the data gathered by the sensor seemed to be noise caused by the light, which can be seen in this figure based on how much noise there is during the on part of the light cycle compared to the much smoother line during the off part. But still nothing that would suggest oxygen being produced in the vial.

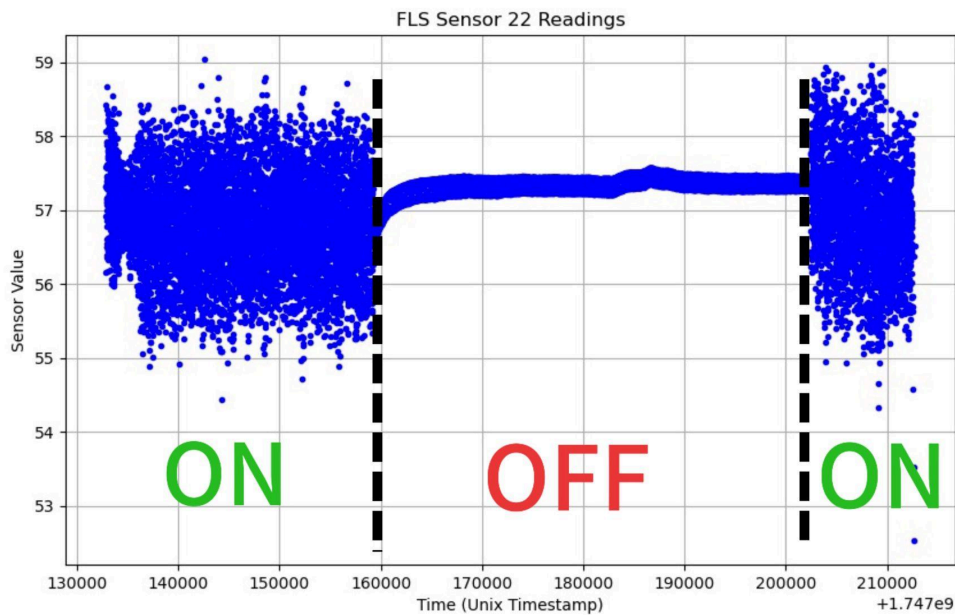


Figure 15: A graph generated from the measurements gathered during the experiment using 50W LED light and the stronger pump, showcasing ON and OFF parts of the 12:12 light cycle

This experiment showed a new issue with the new setup. The LED light was causing the noise and inaccurate readings from the sensor. A possible explanation for this could be that this specific light operates directly off AC with half-wave rectification built into the circuit, which means that for half AC wave, the light is on and for the negative part, it is off, which is why it flickers at the frequency of 50Hz, which in turn was causing the noise and inaccuracies. This error can not be seen with a human eye, however, it can be easily detected when pointing a phone camera at the light. However, this does not mean that all LED lights designed with a pulse waveform cause such flickering.

3.2.2 Future steps

There are still some steps left not included in the scope of this thesis, which would make this experiment a true success and make it its most optimal version.

The most relevant and important addition would be a full calibration of the sensor so that the readings would be directly translated into the partial pressure of oxygen. For this, in addition to the already built setup, monitoring of the temperature and the air pressure in the room would be necessary.

An ideal culture mixing method for this setup would be to use magnetic stirrers, which can be gentler and mix the liquid more uniformly.

In the scope of this thesis, ensuring the sterility of the environment was not critical; therefore, it was done on a very surface level. However, when further developing and running the full experiment again, it would be critical to sterilize all the components.

Furthermore, to have as controlled an environment as possible, while maintaining proper darkness when cyanobacteria are not photosynthesizing, would be to keep the experimental setup in an enclosed space, as seen in the original setup (Figure 2)

A good addition for remote monitoring and control of the system would be to use a small camera that could capture not just the stability of the system, but also observe the growth of the cyanobacteria inside the vial.

At the moment lights are positioned optimally; however, sometimes even the smallest movements put them in a spot where they directly affect the measuring system of the sensor, therefore, adding undesirable noise to the measurements. For future steps, a more permanent solution for the lighting system is needed.

On top of that, for aeration, a more advanced control system could be implemented, which would take the CO₂ levels inside the vial into consideration and pump the air in based on that.

SUMMARY

This thesis presents the design, development, and implementation of an automated experimental setup to measure Oxygen partial pressure inside the vial with cyanobacteria, more specifically *Synechococcus*. The goal was to create a remotely controllable platform capable of monitoring and regulating environmental parameters such as lighting and aeration, while also capturing biological activity through optical oxygen sensing.

A luminescence-based optical oxygen sensor, developed at Tartu Observatory, was used to measure the partial pressure of oxygen inside a closed vial. The data gathered was used to validate the setup by relating the measurements to the known biological processes.

A Raspberry Pi 5 was used as the central control unit, enabling remote monitoring and automation through programmable control loops. Environmental variables were regulated with the use of a relay board, and for overall stability and safety, 3D-printed mounts and connectors were implemented.

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