Designing an Open-hardware Remotely Controllable Phototurbidostat for Studying Algal Growth

Gervasi Alain
University of Liege
BotaBotLab, B22
Chemin de la Vallée, 4
4020, Liege, Belgium
A.Gervasi@ULiege.be

Cardol Pierre
University of Liege
Phytosystems, B22
Chemin de la Vallée, 4
4020, Liege, Belgium
Pierre.Cardol@ULiege.be

Meyer Patrick E.
University of Liege
Biosys, B22
Chemin de la Vallée, 4
4020, Liege, Belgium
Patrick.Meyer@ULiege.be

ABSTRACT

Keeping an algal culture at a constant turbidity requires expensive and complex devices. We designed a low-cost, user friendly but also highly configurable phototurbidostat using 3D-printing, open-source software and electronics. The device is able to monitor in real time a culture in photobioreactor, and dynamically adjust the conditions to maintain the turbidity at a desired value. It can accommodate to a large set of volumes or laboratory equipments with little effort thanks to its modular and scalable design. Each module (such as light, pumps or sensors) are autonomous and controllable via Wi-Fi. Furthermore, our phototurbidostat is fully open-source and can be remotely controlled by a smartphone or a computer via a web-based graphical user interface. Finally, the device can be reproduced easily for a cost ranging between 1/20th and 1/50th of the price of a classical commercial device.

CCS Concepts

• Hardware→Communication hardware, interfaces and storage→Sensors and actuators.

Keywords

Arduino; Bio-automation; Node-red; Open-hardware; Raspberry Pi; Wemos.

1. INTRODUCTION

Open-source hardware is hardware whose source files, schematic and code are publicly available for anyone to use, remanufacture, redesign, and resell [1]. This conception is closely related to the DIY and "Maker" movement and analogous to the open-source software movement. The growing accessibility of 3D printing and electronic components allow to bring this concept to the academic research and has led to the emergence of open-source, easy to use and very inexpensive laboratory devices. Researchers can now build hardware for a fraction of the cost of a commercial one. Maintenance and upgrades of this hardware also become more accessible. They are also able to perform new experiments thanks

Permission to make digital or hard copies of all or part of this work for personal or classroom use is granted without fee provided that copies are not made or distributed for profit or commercial advantage and that copies bear this notice and the full citation on the first page. Copyrights for components of this work owned by others than the author(s) must be honored. Abstracting with credit is permitted. To copy otherwise, or republish, to post on servers or to redistribute to lists, requires prior specific permission and/or a fee. Request permissions from Permissions@acm.org.

ICCBB '19, October 17-19, 2019, Nagoya, Japan

© 2019 Copyright is held by the owner/author(s). Publication rights licensed to ACM.

ACM ISBN 978-1-4503-7681-5/19/10...\$15.00

DOI: https://doi.org/10.1145/3365966.3365969

to the open and modular architecture of their hardware.

The phototurbidostat that we present in this article is a good example of the application of this ideology. A turbidostat is a continuous microbiological culture device, with a feedback between the cell density of the culture (assessed by the turbidity) and the dilution rate [2], [3], [4]. The equilibrium is reached when the growth rate matches the dilution rate. This type of tool is useful for maintaining a culture during a long period or for evaluating the growth rate of a culture. While other laboratories have been able to manufacture DIY turbidostat and other 3Dprinted equipments [5], [6], [7], those devices are not designed for algae cultures since they do not handle light. Our device integrates a lighting module (hence the name phototurbidostat) and can also handle larger volumes of culture by using classical glassware, thereby further reducing the cost. It uses electronic components and software that are easy to use and program allowing any researcher or hobbyist (without prerequisites in electronics or programming) to manufacture easily a modular device at a low cost and capable of performing experiments autonomously while providing a high level of control over the growing conditions. The turbidostat bring forward in this article has been tested on two model species of unicellular photosynthetic eukaryotes. The device uses 3D-printed part, Single Board Computer, microcontroller and electronics modules. More information about the required equipment, the assembly guide and the code are available on the website: www.botabot.uliege.be

2. MATERIAL AND METHODS

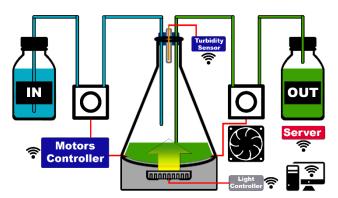


Figure 1. Structure of the turbidostat.

The "Control board" controls the two pumps. The left pump is able to add fresh medium, while the second one can recover the culture. The control board is connected to the "Server" by Wi-Fi. The server recovers the reading of the sensors and send the commands to the modules while displaying a graphical user interface (GUI) to any computer or smartphone connected on the

same network. The turbidity sensor is located on the plug of the Erlenmeyer. The light ring is placed under the Erlenmeyer, illuminating the culture and serving as measurement light for the turbidimeter. The Erlenmeyer and the light are placed on a shaking table, while the rest of the electronic is confined in an MDF (medium density fiberboard) box placed beside the shaker. A fan is placed at the height of the Erlenmeyer to allow dissipation of the heat produced by the light absorption and keep the culture at room temperature.

2.1 Hardware

2.1.1 Microcontrollers

Two families of microcontrollers are used: Atmel [8] (Arduino) and Esspressif [9] (Wemos).

The main board is an Arduino Mega which offers enough free GPIO (general purpose input output) to control the motors and sensors. To facilitate the connections, the Arduino was equipped with a RAMPS 1.4 (RepRap Arduino Mega Pololu Shield) [10] CNC shield (which is typically used to convert an Arduino Mega into a 3D-printer motherboard). It integrates 5 slots for stepper drivers, adds a 128x64 LCD display with rotary encoder, SD card reader and buzzer.

Depending on the requirements, the sensors and external modules are using Arduinos or Wemos boards. A wide variety of sensor modules and easy-to-use library already exist for the Arduino boards (such as Arduino Nano and Pro mini [11]). Their pinout is versatile and they use 5V logical levels. Unfortunately, these boards lack of Wi-Fi support and thus always require a physical connection to communicate with the main server. To overcome this issue, we used the ESP8266 boards (Wemos D1 Mini [12]), which are similar microcontroller. They can be programmed on the Arduino IDE and are compatible with most of the Arduino libraries and sensors but they integrate a Wi-Fi module and therefore, can exchange information via the MQTT protocol [13]. The logical levels of this chip are in 3.3V and there is only one analog pin which can be problematic for some sensors (like the turbidity sensor which is less sensitive on the 3.3V analog pin compared to the 5V on the Arduino). By using widely accessible tools our device is not only more robust but also easily reproducible.

2.1.2 Microprocessors

We use Orange Pi Boards which are generally more powerful and less expensive than the well-known Raspberry Pi while remaining broadly compatible with the Pi softwares. However, our software is compatible with any board. We have tested it on the Raspberry Pi 3B+, Zero and Zero-W on Raspbian (Debian) and on the Orange Pi Win-Plus, Lite, One-Plus, Zero, Zero-Plus, and 3 running on Armbian (Ubtunu).

2.1.3 Culture flask

The culture container was a 2L Erlenmeyer. The volume of the container is not important because its structure is not modified. All the electronics are either separated from the culture container or are located in the plug. We obtained the same results with an Erlenmeyer of 2L or 3L. The culture container is sealed with a sterilizable rubber cap (Deutsch & Neumann 47-55-40mm rubber stopper) including 4 holes. Two of the holes allow two glass rods to pass through the plug to add and remove medium. Another hole in the center allows insertion of a test tube containing the turbidity sensor. The last hole allows the addition of a glass tube topped with a cotton filter that allows gas exchanges with the outside while preserving sterility. Hence, the only requirement for the

glassware is a size large enough to hold a rubber cap that can be perforated with 4 holes. The use of an Erlenmeyer allows to easily obtain a homogeneous culture and a good gas exchange while having a limited light gradient by simply placing the culture on a classical shaking table (Heidolph Instruments Unimax1010 shaker).

2.1.4 Power supply

The power supply is a 500W computer ATX supply which produce the 12V required by the motors and the 5V for the electronics. It is however possible to use less powerful power supplies as long as they output 5V and 12V (the minimum power required is around 15-20W).

2.1.5 *Pumps*

The device uses two peristaltic pumps; one is used to add fresh medium and the other to extract the culture. The two pumps work together to maintain the turbidity and the volume constant. The pumps are composed by Nema17 stepper motors and the body is 3D printed in ABS. This approach allows to have a very cheap but still precise pump (up to $1/10^{th}$ ml of precision) while preventing any contamination (since the liquid is never in direct contact with the mechanism of the pump). The usage of stepper motors instead of DC motors enables a finer control of the speed and number of revolutions. They are also easier to control and calibrate because of the five stepper drivers available on the RAMP 1.4 board.

2.1.6 Pump calibrator

We used a laser (Hyelesiontek 650nm 5mW laser) and a GL5528 photoresistor to create a calibration module for the pumps. This module is a simple liquid level sensor which allows precise calibration of the peristaltic pump. As the 3D printing design of these pumps have some variance and the length/diameter of the silicone tubing can vary, we cannot use a unique ml/turn value for all the pumps. The universal sensor is intended to be placed on a graduated cylinder or a volumetric flask at a specified height (for instance at the mark on a volumetric flask). The volume and the pump calibration should be specified in the GUI. When a calibration is started, the program will activate the pump and count each rotation when filling the flask until the liquid cuts the laser beam, meaning that the volume is reached. The specific ml/turn value is then given by the following formula:

$$Calibration\ Value = \frac{calibration\ volume}{number\ of\ turns}$$

This sensor can be used with any graduated transparent laboratory equipment or pump as long as it is possible to monitor the number of rotations of the rotor. As this robot use NEMA17 stepper motor we can achieve a better resolution (up to 1/200th of rotation) since these motors have 200 steps per revolution and are numerically controlled by the Arduino Mega.

2.1.7 Lighting system

The light is provided by a ring at 96 WS2812B [14] RGB LED also called NeoPixel. These LED are composed of three diodes and one control circuit which allow controlling each primary color at 256 brightness levels and cascading the signal to other LED in the circuit. No matter the number of LED connected in series, we can set a specific color for the first LED with one data wire, a different color for the second, and so on. In our case, the 96 LED are usually set to the same color and intensity in order to get a homogenous lighting. However, it is possible to program a gradient of light or intensity, modify the shape of the LED panel (create a rectangular matrix instead of a disk) or increase the

number of LED on the panel. The LED are controlled by a Wemos D1 mini which get the commands by MQTT.

2.1.8 Turbidimeter

The main sensor currently used in the turbidostat is a turbidimeter composed by a GL5528 photoresistor [15]. The electrical resistance of this component is proportional to the light intensity. The photoresistor is placed in the plug perpendicular to the liquid. As the light comes from the bottom of the photobioreactor, it has to pass through a constant optical path on the liquid to reach the sensor. As a result, the light intensity measured is related to the density of the culture (LED-based system). This approach which gives an absolute analog value on the Arduino (from 0 to 1023), is highly dependent on the culture condition (volume, colors of the culture, color of the light, shape of the Erlenmeyer) and needs to be calibrated in order to match an actual turbidity standard. For our use, a calibration is not mandatory, as we only need to measure if the turbidity increases or decreases compared to a desired value which is typically relative. We tested different approaches involving a 5mW 650nm laser (laser-based system), infrared diode and photodiode or external sensor but these methods requires either a modification of the container or are disrupted by the agitation. To ensure the relevance and the reliability of the LED-photoresistor system, the efficiency was compared to the laser-photoresistor.

The LED-photoresistor approach is therefore the easiest and most affordable. A high light intensity is however required to obtain a strong signal. To limit the actinic effect of the detecting LED (e.g. high light-induced loss of photosystem II activity [16], [17]), short light pulses at max intensity are emitted during 3 seconds every 30 minutes. The rest of the time the lighting is adjusted to the optimal growth intensity for cultivated algae.

2.2 Software Tools and Consumables

2.2.1 3D printing

The 3D models were designed on fusion 360 and printed with a PrusaI3 MK3 on translucent ABS plastic. The ABS is more difficult to use because of its high melting temperature (250 $^{\circ}$ C) and high sensitivity to temperature variation during printing (the printing bed has to be heated up to 90 $^{\circ}$ C to prevent any warping). However, ABS is much more resilient and robust than PLA and has a much higher temperature resistance making it more reliable for scientific applications.

2.2.2 Basic control

The Arduino sensors can be directly controlled via a USB serial connection. This feature allows to easily debug the Arduino program or to use it for a different task. For instance, to get a measurement from an Arduino analog sensor, the Arduino just have to be connected to a computer by USB. The communication protocol is rather similar to Gcode [18] already used by CNC devices. To get the measurement, the string "A1" (for Analog sensor n °1) is sent. When the Arduino receive the command, five analog measurements are performed and the mean of these five measurements is sent back through the serial.

The Wemos are working according to the same principle, except that the command is sent by MQTT on WiFi instead of serial by USB.

As explained previously, the ESP8266 chip integrates a Wi-Fi module but lacks of library compatibility and the GPIO logic levels are lower than the Arduino. In order to control all of our modules by Wi-Fi, given that the Wemos are not fully compatible

with all the sensors, we combined a Wemos with an Arduino. The Arduino is thus responsible for the measurement and the Wemos is responsible for the Wi-Fi connection via MOTT.

The 3 principles of operation are summarized in Figure 2.

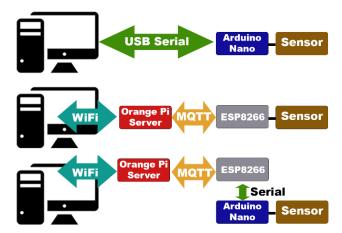


Figure 2. Communication Protocols.

2.2.3 Advanced and autonomous control

The basic control described in the previous section can quickly become overcomplex when several sensors and motors must be autonomously controlled. As a result, we developed a web-based user interface on Node-Red to get, store and analyze the data from the sensor and to control the pumps and light (Figure 3). Node-Red is a flow-based programing tool built on Node.is. Originally developed by IBM on apache license2.0. this software widely used in home automation is used for wiring together hardware devices, APIs and online services as part of the Internet of Things [19]. The web-based interface allows control of the robot with any web browser from any device (computer, smartphone, ...) without having to install or setup any app. It is therefore possible to start an experiment and monitor it via a smartphone from anywhere in the world. For security reasons, it is possible to add a log in page to prevent any undesired connection. We can also add a camera monitoring system in the same GUI.



Figure 3. Main user interface on Node-Red.

The GUI (Figure 3) display the graph of the turbidity; this graph can be saved and exported (as a JSON file) to be used with a different software. The last analog measurement (turbidity) is presented in a gauge and a button allows to measure the turbidity in real time. Below the gauge we have all the information about the dilution factor in the turbidostatic mode. There is also a graph which records all the dilution in order to estimate the division rate

of the cells in the culture. All these data can be saved and exported.

Our interface integrates several controls on the right which allows the user to turn on or off the power supply and turbidostat mode, set the light intensity (for the red green and blue light), set the desired turbidity, set a dilution threshold, set the volume of dilution or manually activate the pumps.

To ensure that the setup is correctly functioning, the bottom right of the GUI is dedicated to monitor the status of the device. The color-coding system used is fairly intuitive: green when the different parts are on and correctly functioning and red if some setups are missing (such as pumps calibration). Further information are available on the website: www.botabot.uliege.be

2.3 Device Control

Three different tabs can be found in the GUI which allows to interact with the photobioreactor. The first one is the "Turbidimeter" which is the main tab used to monitor and adjust the machine. The second is "Calibration" which is used to calibrate the pumps. The calibration should only be performed once or when the length and/or diameter of the silicone tubes has been changed since the system automatically load the previous calibration data. Note that it is also possible to load a saved calibration data by using the option "load calibration". The third one is "Camera" which allows access to the video streams if a monitoring device is added. By default, the system is expecting to receive a MJPEG video stream.

Before starting a culture, it is essential to calibrate the pumps. Silicone tubes cut to the right length must be installed on the pumps. The calibration module is located in the control box. The laser probe must be placed on a laboratory container that is graduated and transparent to a defined level (example: the gauge line of a volumetric flask). The volume of the container can then be entered in the "Calibration" tab on the graphical interface. The input of the pump can be placed in the liquid to aspirate (preferably the same density/viscosity as the liquid that will be used during cultivation) and the output in the graduated container. The calibration will be successful when the liquid interrupts the laser beam.

To start a sterile culture, the following material is sterilized by autoclaving: 2L-erlenmeyer filled with 800 ml culture medium, and cap with silicone tubes already connected to "In" and "Out" bottles. Under a laminar flow hood, the cap is removed to add the algae to the Erlenmeyer. The cap is then be replaced, sealing the photobioreactor, which is placed on a shaker. The LED ring is also placed on the shaker under the Erlenmeyer separated by a glass plate. The silicone tubes are inserted into the 2 peristaltic pumps in the control box next to the agitator. The photoresistor is then inserted into the test tube placed in the center of the cap. The power supply is finally turned on and the monitoring camera adjusted if necessary. No further physical interaction with the photobioreactor is required. Everything is now controlled via the graphical interface.

The main tab allows to setup the volume of the culture (to calculate the dilution factor) and the intensity of the light (the red, green and blue being individually adjustable). The turbidimeter mode can then be activated. If activated, it is necessary to adjust the desired turbidity, the trigger threshold and the dilution volume. Finally, the agitator can be turned on and a first manual turbidity measurement have to be performed in order to trigger the

automatic measurement (by clicking on the "Get turbidity" button on the GUI).

After manually starting the first measurement, the others will then be automatically performed every 30 minutes (default time) as long as: Current Turbidity < (Desired turbidity + trigger threshold).

It is also possible to click on the get turbidity button any time to have a real-time measurement of the turbidity.

If: Current Turbidity > (Desired turbidity + trigger threshold) the turbidostat state rectangle will change from green to blue to announce that the turbidostat switched to regulation mode.

The pump **OUT** will activate and remove "**dilution volume**" followed by the pump **IN** which will add the same volume of fresh medium.

After one minute (the time chose for the solution to homogenize), a **new turbidity measurement** is performed to start a new measurement loop.

When **Current Turbidity** < (**Desired turbidity** + **trigger threshold**), the sum of all dilutions is calculated and the dilution factor, dilution date and time are displayed on the GUI and on the log graph. The measurements will be repeated every 30 minutes until the set turbidity is again exceeded.

Sampling frequency, dilution volume and trigger threshold can easily change: a high sampling frequency (less than 30min) means that the pumps will be activated more often (but with lower volumes) and, therefore, produce a larger amount of monitoring data.

On the contrary, a low sampling frequency and high trigger threshold and dilution volume will activate the pumps less frequently and generate less log data and thus, leading to a rougher turbidity regulation. Wherefore, these parameters should be adjusted according to the needs and growth rate of the algae under study.

2.4 Biological Experiment

2.4.1 Validation of the turbidimeter

To ensure the reliability of the measurements, a serial dilution of a *Euglena gracilis* culture has been performed (with a ¾ dilution between each sample). The OD at 750nm has been measured with a spectrophotometer (UVmc ², Safas, Monaco) and compared with a the turbidimeter present in the photobioreactor with the same 1cm optical path cuvettes (Figure 6 and 7). Two type of light sources were used, a 5mW 650nm red laser and an RGB Neopixel LED.

The values produced by our device are absolute analog measurements ranging from 0 to 1023. After subtracting the value of the blank from all samples, the measurements express the relative absorbance of the solutions and therefore do not correspond to a conventional absorbance measurement [20].

To be able to compare the measurements made by the two turbidimeter, it is required to draw the linear regression line for the two devices. To this end, it is needed to calibrate the two devices with the same concentrations and include the origin on the graph. The slopes of the lines allow to compare the two devices and calibrate one with the other (Figures 6 and 7). Once done, the absolute analogic values can be converted to corresponding absorbance measurements from the spectrophotometer.

The conversion ratio between the two devices is calculated by the formula:

$$\frac{Slope\ (Fig.\,6)\ DIY\ turbidimeter}{Slope\ (Fig.\,7)\ commercial\ turbidimeter} = Conversion\ ratio$$

Consequently, the absorbance at 750nm obtained by commercial spectrophotometer can be calculated using the general formula:

$$\frac{X}{Conversion\ Ratio} = A$$

Where X is the value obtained by our turbidimeter an A will the theoretical absorbance on the commercial spectrophotometer.

2.4.2 Growth monitoring

The first experiment is a validation step to ensure the reliability and accuracy of the turbidimeter. The aim is to ensure that the machine is able to measure low and high cellular concentrations with as little noise as possible and to clearly visualize the latency, exponential and stationary growth phase.

A culture of *Chlamydomonas reinhardtii* has been performed in 800ml of TAP medium [21]. The lighting was adjusted to 15% $(100\,\mu\text{E m}^{-2}~\text{s}^{-1})$ and the agitation has been set to 100RPM (Heidolph Instruments Unimax1010 shaker).

Since the turbidostat mode was not activated, no parameters had to be adjusted in the GUI. The sampling rate was set to 30 min. The measurements were then presented on the graph in the graphical interface. A replicate of this experiment was then performed with another algal species, *E. gracilis* in TAP medium.

2.4.3 Turbidity regulation

The second experiment tested the turbidimeter mode. The aim was to keep the turbidity and volume of a culture constant while recording all the dilution events.

To do this, a *E. gracilis* culture has been set up in 800ml of TAP medium [21]. The light intensity has been adjusted to 50% and the agitation to 100RPM. The culture grew for 1 week until it reached the stationary phase.

The trigger threshold has been set to \pm 5 relative units and a dilution volume of 10ml. Turbidity was kept constant for one and half day, then reduced again for one more day. The volume of the culture has been measured to ensure that the volume remained the same after many dilutions (Figure 10).

3. RESULTS

3.1 Hardware

All the modules were proved to be functional:

The pumps can be effectively calibrated with the calibration module and controlled by the graphical user interface from a computer or a smartphone. The use of a VPN allows remote control of the machine from anywhere in the world. The volumes delivered by the pump are precise and no backflow phenomena were observed.

The light is stable and homogeneous. No overheating problems were observed for the LED ring or the electronic components.

Communication via Wi-Fi is stable and latency-free (below 1000ms). No interference phenomena were observed between the ESP8266 modules even when all the modules are used simultaneously.

The power consumption of the device is around 15W.

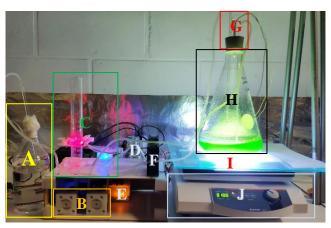


Figure 4. Phototurbidostat installation.

Figure 4 shows the phototurbidostat installation set in turbidostat mode. The installation follows the structure presented in Figure 1. The "IN" (A) and "OUT" (D) bottles are connected to the culture flask (H) through the peristaltic pumps (B) controlled by the Arduino motherboard (E). The LED ring (I) and the culture flask are placed on the shaking table (J). The pump calibration system (C) and the fan(F) are located on top of the MDF control box. The turbidimeter (photoresistor) is in the rubber plug (G).

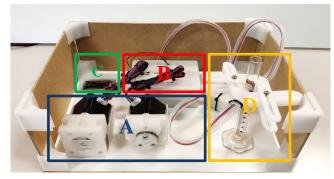


Figure 5. Control box.

The control box presented in the Figure 5, measures 32 x 21 x 11 cm. The modules; pumps (A), motherboard (B), Wi-Fi module(C) and pump calibration module (D) are not permanently fixed in the box and the MDF panels are removable allowing easy modifications inside the box.

3.2 Turbidimeter vs Spectrophotometer

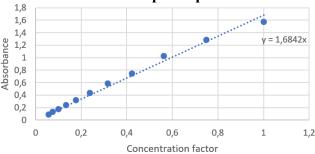


Figure 6. E. graclilis OD 750nm.

Figure 6 shows the variation in absorbance at 750nm depending on the concentration factor of the of the algae solution (*E. Gracilis*) while Figure 7 present the photoresistor analog reading obtained with the LED-based system with the same solution and optical path.

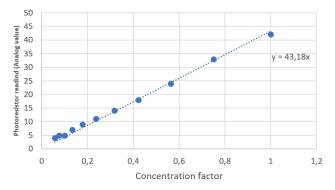


Figure 7. E. graclilis OD Neopixel LED.

As can be seen from Figures 6 and 7, the measurements between the commercial spectrophotometer (Safas Monaco UVmc 3) and the DIY spectrophotometer using a Neopixel LED are different but both give a linear slope. The slope from the commercial spectrophotometer is 1.68 and the one for the DIY spectrophotometer is 43.18. The conversion ratio of both devices is therefore: 25.7

We can test the validity of the ratio using one measurement. Here the concentration factor at 0.8x was used and gave the relative absorbance of 33. Once converted give:

$$\frac{33}{25.7} = 1.2840$$

The equivalent measure done with the commercial spectrophotometer gave the value 1.2847. We can therefore conclude that the DIY sensor is suitable for precise measurement and the value obtained can, after calibration, be converted in absorbance unit.

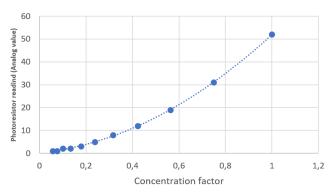


Figure 8. E. graclilis OD 5mW 650nm laser.

As can be seen from Figure 8; the laser-based system does not produce a linear graph. This can be explained by the wavelength of the laser, which is at 650nm and the range of absorption of *Euglena* (in the visible from 380 to 600nm and from the 600 to 720nm in the near red) [22]. The values measured are not only due to turbidity because the 650nm light of the laser-based system is affected by the absorption of the chlorophyll [23], [24]. Which is not the case for the commercial spectrometer because it is set at 750nm which is out of the range of absorption of *Euglena*. It would be possible to use a 5mW 750nm laser, but this one would still be disturbed by the agitation of the liquid. The LED-based system is therefore the best solution. This system works regardless of the agitation and covers all the visible spectrum. No noticeable impact on the reliability of the measurements caused by absorptions phenomena have been observed.

3.3 Growth monitoring

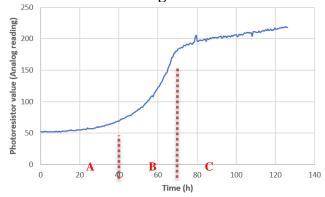


Figure 9. Growth curve of C. reinhardtii culture.

The tree main phases of the culture are easily distinguishable in Figure 9; "lag" (A), "log" (B) and "stationary" phases (C). Almost all measurements match the theoretical curve expected from an algal culture aside from t=79. The overall noise is relatively low although the noise gets slightly higher with high turbidity. We can therefore conclude that the homemade turbidimetry system seems suitable for real-time biomass estimation.

3.4 Turbidostatic Function

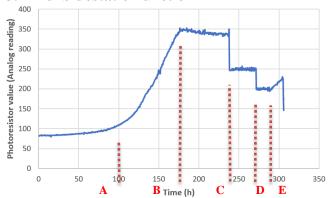


Figure 10. Turbidity regulation of E. graclilis culture.

The *Euglena* culture (Figure 10) has grown from the "lag phase" (A) to "exponential phase" (B) until the maximum turbidity value of 350 was achieved (C) (Figure 10). A first command was set to check if the system can keep a culture at 250 (arbitrary value). We observe that the regulation system starts and the turbidity quickly drop to 250 and stabilized (D). After one day and half, the turbidity was set at 200 (E), and the device was able to maintain the turbidity regardless of the command. Once the turbidostatic mode turned off (F), the culture has resumed its growth.

4. DISCUSSION

From a technical perspective, the device successfully fulfills its functions. The 3D printed and automatically calibrated pumps are reliable and also accurate, the graphical interface works smoothly on a computer as well as on a smartphone, the remote-control function and the video monitoring are both correctly functioning. The turbidity sensor has given similar values in reference to the commercial spectrophotometer and proved its reliability for measuring the growth of algae and ensuring the feedback system.

This research focused on the reliability and precision of the device to achieve its functions before analyzing more in-depth biological experiments involving algae cultures. Few species of algae have been used and more biological experiments will be performed.

Other sensors could be integrated such as a pH, temperature or oxygen sensor. Their integration should be easy thanks to the "plug and play" approach used for the conception of this device.

5. ACKNOWLEDGMENTS

We would like to thank Haval Abdulla and Manuel Noll for their help and support during the writing process and Nicolas Juste for his participation in the device development during his internship.

6. REFERENCES

- [1] Alicia Gibb. 2015. Building Open Source Hardware: DIY Manufa [1] cturing for Hackers and Makers. Addison-Wesley. 253–277.
- [2] David Moore, Geoffrey D. Robson and Anthony P. J. Trinci. 2011. 21st Century Guidebook to Fungi. *Cambridge University Press*. 470-472. DOI= https://doi.org/10.1017/CBO9780511977022
- [3] Winder, C. L. and Lanthaler, K. 2011. The Use of Continuous Culture in Systems Biology Investigations. *Methods in Systems Biology*, 261–275. DOI= 10.1016/b978-0-12-385118-5.00014-1
- [4] Sorgeloos P, Van Outryve E, Persoone G and Cattoir-Reynaerts A. 1976. New Type of Turbidostat with Intermittent Determination of Cell Density Outside the Culture Vessel. *Applied and Environmental Microbiology*. 31 (3): 327–331. PMID = 16345153.
- [5] Chris N. Takahashi, Aaron W. Miller, Felix Ekness, Maitreya J. Dunham and Eric Klavins. 2014. A Low Cost, Customizable Turbidostat for Use in Synthetic Circuit Characterization. ACS Synth Biol.4(1), 32-38. DOI= 10.1021/sb500165g
- [6] Pilizota, T. and Yang, Y. 2018. "Do It Yourself" Microbial. Cultivation Techniques for Synthetic and Systems Biology: Cheap, Fun, and Flexible. Frontiers in Microbiology, 9. DOI= https://doi.org/10.3389/fmicb.2018.01666
- [7] Hoffmann SA, Wohltat C, Müller KM and Arndt KM. 2017. A user-friendly, low-cost turbidostat with versatile growth rate estimation based on an extended Kalman filter. *PLoS ONE* 12(7). DOI= https://doi.org/10.1371/journal.pone.0181923
- [8] 8-bit Microcontroller with 4/8/16/32K Bytes In-System Programmable Flash. 2008. Retrieved May 10, 2019 from https://www.sparkfun.com/datasheets/Components/SMD/AT Mega328.pdf
- [9] ESP8266. 2019. Retrieved May 10, 2019 from https://www.espressif.com/en/products/hardware/esp8266ex/ overview
- [10] RAMP1.4. (March 2019). Retrieved May 10, 2019 from https://reprap.org/wiki/RAMPS_1.4

- [11] Arduino products. 2019. Retrieved May 10, 2019 from https://www.arduino.cc/en/Main/Products
- [12] A mini wifi board with 4MB flash based on ESP-8266EX. 2018. Retrieved May 10, 2019 from https://wiki.wemos.cc/products:d1:d1_mini
- [13] Hunkeler, U., Truong, H. L., and Stanford-Clark, A. 2008. MQTT-S — A publish/subscribe protocol for Wireless Sensor Networks. 3rd International Conference on Communication Systems Software and Middleware and Workshops (COMSWARE '08). DOI= 10.1109/comswa.2008.4554519
- [14] WS2812B Intelligent control LED integrated light source. Retrieved May 10, 2019 from https://www.kitronik.co.uk/pdf/WS2812B-LED-datasheet.pdf
- [15] CdS PHOTOCONDUCTIVE CELLS GL5528. Retrieved May 10, 2019 from https://pi.gate.ac.uk/pages/airpifiles/PD0001.pdf
- [16] H. Tschiersch, and E. Ohmann. 1993. Photoinhibition in Euglena gracilis: Involvement of reactive oxygen species. *Planta*. 191(3), 316–323. DOI= https://doi.org/10.1007/BF00195688
- [17] EsaTyystj ärvi. 2013. Chapter Seven Photoinhibition of Photosystem II. International Review of Cell and Molecular Biology. 300, 243-303. DOI= https://doi.org/10.1016/B978-0-12-405210-9.00007-2
- [18] G-code (March 2019). Retrieved May 10, 2019 from https://reprap.org/wiki/G-code
- [19] Heath, Nick. 2014. How IBM's Node-RED is hacking together the Internet of things. Retrieved May 02,2019 from https://www.techrepublic.com/article/node-red/.
- [20] Swinehart, D. F. 1962. The Beer-Lambert Law. *Journal of Chemical Education*, 39(7), 333. DOI= 10.1021/ed039p333.
- [21] Gorman, D.S., and R.P. Levine. 1965. Proc. Natl. Acad. Sci. USA 54, 1665-1669.
- [22] Strother G.K, and Wolken J. 1961. In vivo Absorption Spectra of Euglena: Chloroplast and Eyespot. *The Journal of Protozoology*, 8(3), 261–265. DOI= 10.1111/j.1751-1097.1976.tb06770.x
- [23] Zucchelli, G., Jennings, R. C., Garlaschi, F. M., Cinque, G., Bassi, R. and Cremonesi, O. 2002. The Calculated In Vitro and In Vivo Chlorophyll a Absorption Bandshape. *Biophysical Journal*, 82(1), 378–390. DOI= 10.1016/s0006-3495(02)75402-7/S0006-3495(02)75402-7
- [24] Pareek, S., Sagar, N. A., Sharma, S., Kumar, V., Agarwal, T., Gonz alez-Aguilar, G. A. and Yahia, E. M. 2017. Chlorophylls: Chemistry and Biological Functions. *Fruit and Vegetable Phytochemicals*, 269–284. DOI= 10.1002/9781119158042.ch14