UNIVERSITAT POLITÈCNICA DE VALÈNCIA

ESCOLA TÈCNICA SUPERIOR D´ENGINYERIA AGRONÒMICA I DEL MEDI NATURAL

Assembly, set-up and operation of a laboratory scale 1L photobioreactor

TRABAJO FIN DE GRADO EN BIOTECNOLOGÍA

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Curso Académico: 2015/2016

VALENCIA, Julio de 2016

Title: Assembly, set-up and operation of a laboratory scale 1L photobioreactor.

Abstract: Currently, cyanobacteria are photosynthetic microorganisms that have been considered as potential candidate for the production of biofuels or pharmaceutical compounds among others. For their cultivation, there are specific systems called photobioreactors in which all their natural requirements (light, CO_2 and nutrients) are provided. Moreover, the cultivation of these microbes involves a set of sensitive parameters, such as temperature, pH, mass transfer or mixing, that should be maintained under specific ranges to get the photobiological process stable. These parameters are controlled by a serie of associated devices that conform the photobioreactor system. The assembly and operation of a 1L lab-scale flat-bed photobioreactor and its associated devices using a computer program were performed in this work. Furthermore, a set of calibration procedures and characterization experiments were done to check the proper operation of the photobioreactor system and to collect relevant information for future cultivation processes. This investigation is set inside the European project CyanoFactory (FP7-Energy 308518). This project is carried out by a consortium composed of several European universities and research companies. It pursues the aim of generating photosynthetic microorganisms cultivated in photobioreactors with the capacity of producing biohydrogen.

Keywords: Photobioreactor, cyanobacteria, process engineering, control of process variables.

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Título: Montaje y operación de un fotobiorreactor de un litro a escala de laboratorio.

Resumen: En la actualidad, las cianobacterias son microorganismos fotosintéticos que han sido considerados como fuentes prometedoras para la producción de biocombustibles o compuestos farmacéuticos entre otros. El cultivo de estas bacterias requiere de sistemas específicos denominados fotobiorreactores, los cuales proporcionan los requerimientos que necesita la cyanobacteria en condiciones naturales (luz, CO_2 y nutrientes). Además, el cultivo de estos microorganismos lleva consigo la regulación de una serie de parámetros, como son la temperatura, el pH, la transferencia de calor o la homogeneización, para mantener el proceso fotobiológico estable dentro de unos rangos. Estos parámetros son controlados por una serie de dispositivos asociados que forman parte del sistema del fotobiorreactor. El ensamblaje y operación de un fotobiorreactor de tipo plano de 1L a escala laboratorio y de sus dispositivos asociados usando un programa informático fueron el objeto de este trabajo. Además, se realizaron una serie de calibraciones y experimentos de caracterización para comprobar el correcto funcionamiento del fotobiorreactor montado y para obtener información relevante que debe ser tenida en cuenta para futuros cultivos de cianobacterias. La presente investigación se enmarca dentro del proyecto de la Comisión Europea Cyanofactory (FP7-Energy 308518) conformado por un consorcio de universidades y empresas europeas que tiene el fin de crear microorganismos cultivados en fotobiorreactores que sean capaces de producir biohidrógeno.

Palabras clave: Fotobiorreactor, cianobacterias, ingeniería de procesos, control de variables de proceso.

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List of Abbreviations

"	Inch
ºC	Degrees Celsius
µE	Microeinsteins
µm	Micrometers
AI	Analog Input
AO	Analog Output
Bar	Bar
cm	Centimetre
cm^2	Square centimeter
CO_2	Carbon dioxide
COM	Component Object Model
DI	Digital Input
DO	Digital Output
E	Energy
g	grams
GFC	Gas Flow Controller
GFM	Gas Flow Meter
h	Hour
HCl	Hydrochloric acid
KCl	Potassium chloride
kg	Kilogram
L	Liter
LED	Light Emitting Diode
M	Molar
mA	Microamperes
min	Minutes
mL	Mililitre
mm	Nanometer

NaCl	Sodium chloride
O_2	Oxygen
PAR	Photosynthetically Active Radiation (440-700nm)
PBR	Photobioreactor
PC	Personal Computer
PCI	Peripheral Component Interconnect
PE	Photosyntethic efficiency
PIC	Programmable Interface Controller
RH	Relative Humidity
V	Volt
Vol.	Volume
X	Biomass
YX	Biomass Yield

1. Introduction

According to the last United Nations review of world population prospects (DESA, 2015), a demographic increment in 9.724 million people is estimated by 2050. It means that the world population is growing at a rate of 1.18 per cent each year. This fact has a direct implication in power generation and energy consumption. The limited amount of fossil energy resources, especially petroleum reserves, opens the need to look for new options. In fact, according to the annual world primary energy consumption, the global oil consumption is increasing by 0.8 million barrels per day (BP, 2015). Thus, the quest and the development of new alternative energy sources, as neutral solid, liquid and gaseous biofuels, have intensified nowadays (Bahadar and Bilal Khan, 2013; Singh and Sharma, 2012).

Moreover, a rise in energy consumption lead to an increase of CO_2 emissions in the atmosphere worsening the greenhouse phenomenon, the global warming and the climate change. Thus, there is a need for new global strategies in energy security and CO_2 mitigation. In this way, cyanobacteria have been suggested as a good alternative (Moody, McGinty and Quinn, 2014; Sayre, 2010; Brennan and Owende, 2010). This potential next-generation feedstock could be used for bioenergy and biofuel production. In fact, they are being researched to produce biodiesel or biohydrogen which have been considered the most important future energy carriers (Kwon, Rögner and Rexroth, 2012). Moreover, they offer some potential advantages over previous-generation biofuel feedstock. They help to mitigate carbon dioxide emissions; they do not need arable land, crop rotation and pesticide applications; they require less water use and they do not compete with global food markets (Moody, McGinty and Quinn, 2014). In fact, cyanobacteria have a rapid growth and a high biomass yield, and they also could be cultivated under a wide range of conditions and produce high-value co-products with an enormous variety of industrial applications (Bahadar and Bilal Khan, 2013; Markou and Nerantzis, 2013).

Nevertheless, its cultivation process presents some challenges since it is difficult to optimize the mutually dependent factors and the complex interactions that take place in the photobiological process (they have been explained later). Furthermore, these events also hinder the transition from lab-scale to industrial scale operations. These aspects make the production costs of microalgae culture very high, this being the main bottleneck (Kwon, Rögner and Rexroth, 2012).

Cyanobacteria, are photosynthetic organisms, that under natural conditions, absorb sunlight, assimilate and fix CO_2 from the atmosphere and take water and nutrients from their habitat medium. The microorganism need adequate and stable conditions for their growth and development. The use of light and CO_2 allows them to produce chemical energy and store organic carbon compounds respectively, through a biochemical transformation called photosynthesis (Olivieri, Salatino and Marzocchella, 2013). Moreover, numerous cyanobacteria species are well suited for genetic manipulation. *Synechocystis* sp. PCC 6803 is the most highly studied cyanobacteria during the las decades. It was the first photosynthetic autotroph to have its genome sequenced (Kaneko et al., 1996), and it is being very used in many laboratories around the world (Zavřel et al., 2015).

At present, there are different types of systems for the cultivation of microalgae outside its natural environment. They could be mainly divided into two classes: open cultures and closed photobioreactors (PBRs). The first ones operate in outdoor conditions and are characterized by simple cultivation techniques, lower production rates and reduced operation costs. Nonetheless, one of the main drawbacks of these cultivation systems is the lack of control over the process conditions. The biomass productivity per each area and volume unit depends on the location, the season and further non-controlled environmental factors, such as illumination, temperature, rain, dust or the presence of birds and insects. Moreover, contamination due to other microorganisms that can compete with the cultivated algae strains, can also limit the productivity rate (Bahadar and Bilal Khan, 2013). To overcome these problems new open culture configurations have been researched, as the closed open systems which are covered with a transparent and translucent barrier. Despite these new alternatives, closed photobioreactors are still a better alternative though its expensive development costs.

A PBR is a closed equipment which does not have a physical direct contact with the environment, providing higher efficiency and biomass productivity as well as a better optimization of the photobiological process. Therefore, these systems minimize possible outside contaminations and allow a higher production rate of biofuels and high-value compounds (Singh and Sharma, 2012). Currently, there are several configurations of PBRs with a wide range of designs and applications: tubular, flat plate, bubble column or airlift. The development of suitable photobioreactors should take into account the following factors among others: microalgae strain, light radiance, rates of mass transfer, size and capacity of the reactor, gas mixture, aeration rates, foaming conditions and fouling factor. In this way, each type of PBR was designed according to a specific type of cultivation process. In Bahadar and Bilal Khan (2013) a list of closed photobioreactor systems for the cultivation of microalgae species can be found as well as the advantages and disadvantages of each one.

As already stated, this investigation is set within an European Commission Cyanofactory project (FP7-Energy 308518) whose main purpose is the production of hydrogen by cultures of photosynthetic microorganisms in photobioreactors. Furthermore, *Synechocystis* sp. PCC 6803 is the main microalgae strain under investigation. Thus, considering these details and the information previously detailed, a scale-lab 1L flat-bed photobioreactor system had been chosen for this aim. Bearing out this PBR performance, Janssen et al. (2002) reviewed and summarized possible PBR configurations and remarked that flat-panel photobioreactors are high-performance systems in terms of photosynthetic efficiency (PE > 10 %), biomass yield on light energy (YX/E > 1 g/E) and biomass density (X > 10 g/L).

Synechocystis sp. PCC 6803 is a photoautotrophic microorganism that can perform an oxygenic photosynthesis along with typical prokaryote features. The main requirements for its growth and development are light, CO_2, and nutrients. These requirements have to be provided by the PBR system to control and optimize its growth and the bioprocess. (Vasumathi, Premalatha and Subramanian, 2012; Brennan and Owende, 2010). Consequently, temperature, heat transfer, pH, mass transfer, power and mixing are sensitive parameters for the cyanobacteria development that should be taken into account. All these parameters interrelate with each other, so its maintenance in optimal ranges is important for the bacteria

growth and development. Thus, PBR system should be equipped with a set of instruments and devices that control these parameters.

Light availability is one of the central factors that affect photosynthesis rate and consequently, biomass growth, circadian rhythms, gene expression and productivity (Kwon, Rögner and Rexroth, 2012; Lopo et al., 2012). Photosynthesis occurs due to the absorption of light energy in the wavelength range of 400-700nm. Therefore, photosynthesis depends on the irradiance applied to cells. The representation of photosynthetic ratio versus irradiance shows a hyperbolic relationship, in which three phases can be distinguished: activation of the photosynthesis machinery, saturation event and photoinhibition. This hyperbolic relationship depends on each particular cyanobacterium strain. It has been seen that *Synechocystis* sp. PCC 6803 can growth under a white light intensity range of 100-300 µmol photons $\cdot m^{-2} \cdot s^{-1}$ (Lopo et al., 2012). There again, the shape and geometry of the PBR as well as the culture density could reduce the light absorption, causing the appearance of unproductive dark zones and an autotrophic metabolism rate decrease (Acién Fernández, Fernández Sevilla and Molina Grima, 2013).

Cyanobacteria also need CO_2 for its growth, which is taken up in two phases: absorption and fixation, the latter one depending on temperature and the number of available photons. The carbon absorption by microorganisms normally occurs in water. There the transfer of gaseous CO_2 to the medium is influenced by pH, temperature, mixing, area of contact, the partial pressure of CO_2 in air — which is in contact with the aqueous medium — and also the air flow rate. When the CO_2 becomes soluble in water, the most of it will be as CO_2 molecules surrounded by water; but some change its form to carbonic acid (H_2CO_3), which help to acid-base equilibria (pH). Carbonic acid could dissociate into ion bicarbonate and carbonate, transferring one or both protons (H^+) to the medium. The reverse process takes also place, so both chemical events help in pH regulation. Mixing increase, CO_2 concentration in air, pH and the area of contact reduce mass transfer resistance; however, mixing process could lead to more power consumption and cell damage (Vasumathi, Premalatha and Subramanian, 2012).

Production of 1 g of biomass requires of 1.8 g of CO_2 and produces 1.3 g of O_2. High oxygen levels can cause photosynthesis machinery inhibition and severe photo-oxidation in many cyanobacteria species. Hence, mass transfer of O_2 and its desorption is another fact to consider, in terms of finding a comprime for the above-described parameters (Acién Fernández, Fernández Sevilla and Molina Grima, 2013).

Another important point is temperature. Temperature has to be maintained stable between 20-38 ºC for most of the cyanobacteria cultures. Outside this range, a drop of growth rate and even death could be provoked. Furthermore, this factor can influence or be influenced by other parameters, since on the one hand, it modifies gases mass transfer. CO_2 and O_2 are more soluble in cold water, whereas their solubility is very low at 25ºC (Acién Fernández, Fernández Sevilla and Molina Grima, 2013). On the other hand, irradiance has a direct effect in temperature rise. Thus, in this type of photobiological process is usually important to have some refrigeration system. Cell metabolism also produces energy and increases temperature.

Just as temperature, pH could also be affected by irradiance, so that high irradiance increases photosynthesis rate and thereby, pH value, since CO2 consumption causes a gradual rise in pH. An efficient control of pH

range and heat transfer are important to maintain an optimal growth rate and minimize CO_2 losses (Fernández et al., 2010).

Mixing process should also be remarked. It is necessary to increase the mass transfer and generate a turbulence that reduce the nutrient gradient in the culture, avoid cell sedimentation and force the cells to move enhancing the photosynthesis rate and nutrients uptake. Mixing can occur by aeration or agitation using pneumatic or mechanical devices respectively. Both systems can cause cell damage, so for cultivation process it is important to find a trade-off between the turbulence and the cell damage (Acién Fernández, Fernández Sevilla and Molina Grima, 2013).

Finally, nutrition is the last main requirement. Some fundamental organic and inorganic sources, especially inorganic mineral nutrients, should be provided in the medium to reduce lag phase and maximize growth rate (Vasumathi, Premalatha and Subramanian, 2012). BG11 is the culture medium characteristic of *Synechocystis* sp. PCC 6803.

Hence, this overall parameters and requirements as well as their relationships should be taken into consideration in both PBR design and the accomplishment of natural environmental conditions for the cultivation process. See visual major factor relationship diagram of Acién Fernández, Fernández Sevilla and Molina Grima (2013). In the light of the above, our 1L scale-lab flat-bed photobioreactor system has a series of components and devices to control all parameters, providing the essential requirements for the bacteria growth and optimizing the photobiological process.

Therefore, the object of this study was the assembly and operation of our 1L scale-lab flat-bed photobioreactor system. The biological knowledge of the cyanobacteria, the requirements interconnection as well as its effects on cultivation and photobiological process were needed for the development of this work. This research was done in a set of steps as it is shown in Figure 1. Firstly, all parts and devices of the PBR system were characterized. Afterwards they were linked up developing the PBR system. Once it was assembled, it was learned to use the computer programs for remote control. Some devices were calibrated according to manuals' instructions and finally, a set of experiments were performed to check that everything works properly and to characterize the PBR operation. These experimental results gave relevant information of how it should operate during real cultivation process.

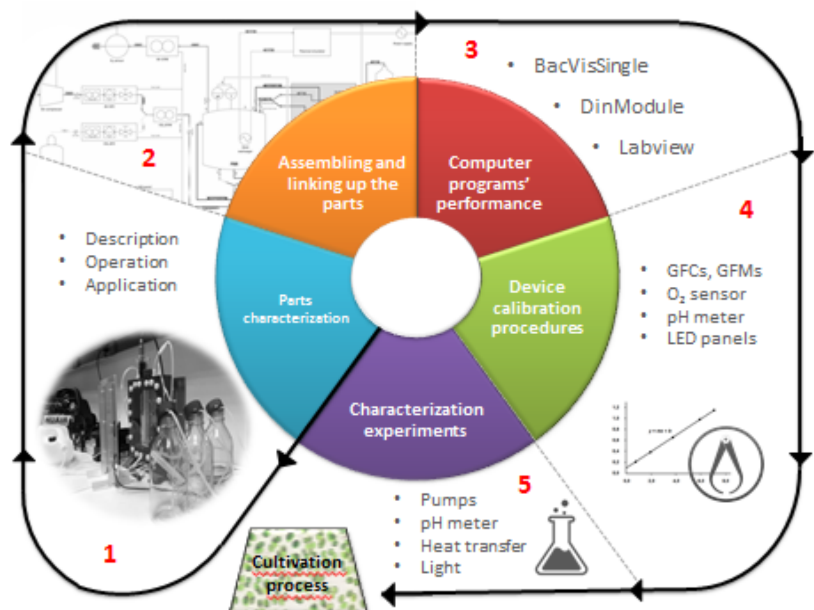


Figure 1. Steps carried out for the execution of this work.

2. Objectives

The objective of this study is the assembly, operation and test of a 1L lab-scale flat-bed photobioreactor system and to compile all the information required for future users of the system. In order to accomplish this purpose, a set of sub-objectives were established:

- Characterization of all the parts, components and devices that make up the photobioreactor system.
- Assembly of the photobioreactor system.
- Determination and verification of the smooth running of the computer programs.
- Determination of the calibration procedures in devices which require it.
- Tests to check the proper assembly and functioning of the photobioreactor system.

3. Materials and Methods

3. 1. Photobioreactor system parts characterization

As mentioned above, a PBR presents a set of components and devices that allow to maintain proper culture cyanobacteria conditions. In this way, our scale-lab 1L flat-bed photobioreactor is composed of the following parts:

A. Flat panel photobioreactor (PBR). It is the recipient which contains the cyanobacteria culture and it is made of a biocompatible polymer. Its measurements are 240x290x40 mm, its capacity is 1.18 L, although its total capacity is 1.32 L, its empty weight is 3,5 kg and it also has a light capturing surface of 661 cm^2. It presents two plastics surfaces on both sides which could take apart unscrewing white nuts to encourage disinfection and cleaning process. For further information see KSD Manual.

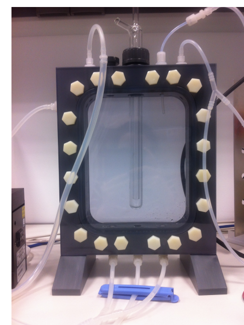


Figure 2. Flat panel photobioreactor.

B. Control Box. It is the key that allows communication between the computer program and the different measurement devices. It contains two PCI cards designed for digital/analog signal conversion and is connected with the following electronic transmitters and components:

- pH-controller with temperature sensor.
- Dose pump for HCl and NaCl (manual / automatic).
- Dose pump to fill and for constant flow rate (manual / automatic).
- Speed controller for dose pump.
- Main switch ON / OFF.
- Analog card - converter - for PC.
- Controller and power supply for LED lights.
- Gas controller and mixer for air and CO_2.
- PC

Figure 3. Control Box opened.

C. LED panels. They provide the artificial light needed for photosynthesis process. The flat panel PBR is flanked on either site by a LED metal panel, two panels in total, encouraging the lighting of the complete culture medium surface in the PRB. Each panel has 84 LEDs in one of the slides, arranged in 4 rows of 21 LEDs. The measurements of the panels are 500x8x1 mm and they irradiate white light between 400-750 nm. The panel radiance intensity can be change but not the emission wavelength. For further information see KSD Manual.

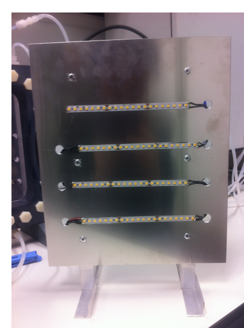


Figure 4. LED panel.

D. Remote control system. It refers to the desktop computer with Windows 7 software. It has three computer programs related to the PBR: Labview, BacVisSingle and DinModule. Labview is the main photobioreactor program for data collection, data visualization and remote control of the photobiological process. This program allows to set up all the parameters of the system and saves all the measurements taken by the overall devices. The other both, BacVisSingle and DinModule, are used mainly for calibration procedure of O_2 sensor and pH meter respectively. Controlling these devices and taking measurements are also possible with both programs.

Figure 5. Computer.

E. Mass Flow Meters (GFMs). GFMs are devices designed to measure and show the gas flow rate (mL·min^{-1}) that passes through them. They do not operate with liquids, only with pure or filtered gases. There are two GFMs in our PBR system. One measures the air that comes out of the PBR. The other one indicates the total amount of CO_2 which enters the PBR after air flow and a percentage of CO_2 were mixed together. Since for photosynthesis and cell growth control, it is important to know how much of each gas is moving into and out of the PBR. For a proper operation, these devices should be collocated in vertical position and they need 30 minutes to warm-up since they were switched on.

Figure 6. Air Mass Flow Meter (GFM).

Related to their operation principles, only a small portion of the total gas stream that enters in the device is used for the measurement. This small portion is shunted through a capillary stainless steel sensor tube. There, the laminar gas flow is heated and carried from an upstream coil to a downstream coil windings. A temperature change is detected electronically and it is dependent of a resistance differential. The GFM provides an output signal that is a function of the amount of heat carried by the gas to indicate mass-molecular based flow rate. For further information see GFM Operating Manual Aalborg.

F. Mass Flow Controllers (GFCs). GFCs are devices that control the gas flow rate (mL·min^{-1}) that passes through them. They do not operate with liquids either, only with pure or filtered gases. There are also two GFCs in our PBR system, one regulates the air that enters the flat panel PBR and the other one, the CO_2 input. These devices allow to control the mixing, aeration and the gas quantity that enters in the PBR. For a proper operation, they also should be collocated in vertical position and they need 30 minutes to warm-up since they were switched on.

Figure 7. CO_2 Mass Flow Controller (GFC).

They have the same operational components as the GFM plus a PIC and a solenoid valve. Related to their operation principles, they firstly make the same type of measurements as the GFMs, generating an output flow rate signal. Afterwards, this signal is detected by a closed loop control circuit (PIC) and compared continuously with the flow rate value selected in Labview program. Deviations are corrected by solenoid valve adjustments to maintain the constant setpoint. Thus, the valve allows to set the gas flow rate to any desired value within the range of 0-500 $mL \cdot min^{-1}$ in the Air GFC or 0-50 $mL \cdot min^{-1}$ in the CO_2 GFC. This valve is closed when the Labview program is not running. For more information see GFC Operating Manual Aalborg.

G. O_2 sensor. This device measures de oxygen concentration that comes out of the PBR in a range over 0,1-25 % Vol. If the O_2 % is know, the photosynthetic rate as well as the cell growth can be determined. It does not operate with gases which contain polymers, silicons, halogens (fluoride, chloride, bromide, etc.), SO_x or H_2S and it needs between 45 min and 1 hour to warm-up since it was switched on. Moreover, the relative humidity of the environment should be lower than 75 % for proper measurements. If the RH increases, the sensor has to be 12 h at 80 ºC in a hot air oven.

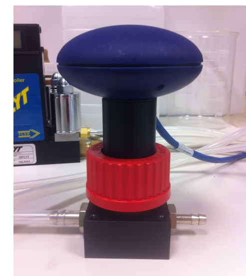


Figure 8. O_2 sensor.

If the red adaptor is unscrewed, there is a sealing ring to avoid possible leaks. There, under the sensor's head, there are two air filters. The external filter can be replaced by another one but not the internal filter. In this case, contact custom service. Regarding its operational principles, it has a oxygen pump cell where gas O_2 molecules are transferred since the cathode to the anode. The sensor correlates O_2 molecules with electrons. Besides, the sensor must not be disconnected from the power supply when it is operating. For further information see the BluesSens BCP-O_2 Manual.

H. pH meter. This electrochemical device measures the pH of the culture inside the PBR. It is unscrewed in PBR port 5 (see Figure 24). Moreover, it has an integrated temperature sensor, which measures the temperature of the culture. These measurements allow to regulate both factors and keep them in a stable range. This electrolyte is filled of a 3M KCl solution. When this solution volume is low, it has to be refilled using the unscrewed black nut which is located on the side of the device. Furthermore, pH meter has a white-transparent cap with the same KCl solution. This cap should be placed at the tip of the pH meter when the device is not being used. For more information see the pH meter Manual.

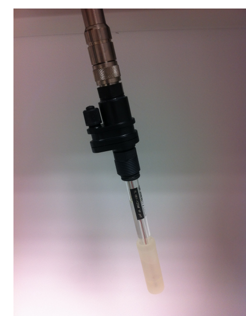


Figure 9. pH meter with the cap.

I. **Piston air compressor Nuair Vento OL 195.** This device takes air from the environment and stores it in the tank until 8 Bar pressure is achieved. At that moment, the compressor stops automatically. Then, the compressed air is released at a specific pressure that can be regulated by the "Pressure regulator". As the air is draining out, the tank depressurizes. When tank pressure reaches its lower limit, the device turns on automatically and stores air until re-pressurizing the tank. For a proper operation, the pressure should be maintained between 2-4 Bar using the "Pressure regulator". The capacity of the air compressor is 6 L and has to be connected to the current supply using the power cable. Furthermore, the silicone tube is connected to the quick metal coupler.

1. On/off switch.
2. Comprised air outlet with quick metal coupler.
3. Operating pressure adjustment knob.
4. Air used pressure gauge.
5. Receiver condensation drainage tap.

Figure 10. Piston air compressor.

J. **CO_2 Cylinder.** This bottles stores compressed CO_2 which can be released using a valve. Since we have not started culture experiments, we have not bought it yet. Because of that, more specific information cannot be provided. This cylinder is required for the cyanobacteria culture due to the fact that these microorganisms need an extra percentage of this gas for photosynthesis process and thereby, for its growth. *Synechocystis* sp. *P*CC6803 needs air enriched with 3 % CO_2 for its culture because the amount of CO_2 that the compressed air has, is not enough.

K. **Schott-Bottles ISO GL45 1L.** There are three 1L glass bottles in our PBR system. Each one stores a specific solution required for the PBR operation. One stores acid solution (HCl) and other, the base solution (NaCl). Both solutions permit the culture pH regulation. The last schott-bottle stores culture medium (BG11) for the culture dilution and renovation. The bottles' volume is of 1L because it has been reported from other researched groups that for a continuous cultivation of the scale-lab 1L flat-bed PBR, 1L volume of this three solutions is enough.

Figure 11. Schott-Bottles.

L. **Waste container.** It is the tank that stores the biological waste, which is the culture residues that overflowed of the PBR. The actual 3 L waste container is only for characterization experiments. For future culture experiments with cells, an adequate container fit for the biological waste collection will be required.

Figure 12. Waste container.

M. Pumps. There are three pumps incorporated into the Control Box but located in the external surface of it. Theses pumps are peristaltic pumps. They have a rotor with a number of “rollers” around the external circumference. Specific flexible silicone tubes are placed inside the pumps, in contact with the “rollers”. The silicone tubes are the carriers whereby the solutions pass through from the Schott-Bottles into the PBR. When the rotor turns, the silicone tubes are compressed and closed by the “rollers”and the liquid between two rollers is forced to move through the tube. This type of peristaltic pumps run at ambient pressure and usually have a range of revolutions to deliver different amounts of fluid. Each peristaltic pump moves a different solution through the tube. The pump on the bottom, is the main pump. It controls the input culture medium flow rate ($mL \cdot min^{-1}$). According to the experimental results, its revolution range is 1-5 $mL \cdot min^{-1}$ (see section 4). The middle pump regulates the input base solution and the last one pumps the acid solution. These two pumps help in pH regulation. They do not have a range of revolutions. On the contrary, they only work at a specific rate revolution (10 $mL \cdot min^{-1}$) (see section 4) and they can only be switched on and off. In addition, for a correct operation of pumps, the Control Box must be placed in vertical position.

Figure 13. Peristaltic pumps on the Control Box surface.

N. Heat exchanger. It is a glass tube to control culture media temperature inside the PBR. It is located in port 3 (see Figure 24) unscrewing the black nut. Water at a particular temperature passes through it, warming up or cooling down the culture’s temperature. The water enters on the top, passes through a thin tube with an open bottom end that is inside the exchanger, flows in the outer layer and finally, exits by the side output. Heat is transferred through the glass wall of the exchanger to the culture. Photosynthetic processes usually require more cooling down because LED panels as well as cell metabolism produce heat, warming up the culture and having negative effects on cell growth and bioprocess development.

Figure 14. Heat exchanger.

O. Thermal circulator. It is a tank that stores water and it can also regulate its temperature. Moreover, it has a pump that forces the water to move from the tank to the heat exchanger and come back. So, it works in a closed system. However, since we do not have this device, more detailed information cannot be given. In addition, plastic tubes will be bought to connect the thermal circulator with the heat exchanger.

P. Other items:

- **Air filters.** They remove solid particles from the air such as bacteria, mould, dust, etc. thanks to their 0.2 µm Millipore membrane. They avoid the introduction of any potential flow impediment in GFCs, GFMs and O_2 sensor, guaranteeing the good functioning of the devices, and they keep sterile culture conditions inside the PBR (cyanobacteria are very sensitive to this). The filter positions in the PBR system are displayed in Figure 22. Moreover they have to be set in a specific position when they are clipped to the silicone tubes. Its location depends on the fluid flow direction. Their blue blind plug side (see Figure 25(D)) is the inlet side whereby the flow enters, and the other one is the outlet side. These filters can be reused 10-15 times, but if an air filter gets wet, it should be replaced by another one immediately. Actually, not all the filters have been placed in their specific positions due to the fact that it is not necessary for the characterization experiments. But for culture experiments, all the air filters have to be located suitably.

Figure 15. Air filter.

- **Valves.** Two types of valves are used in the PBR system: check valves (green color in Figure 16) and Safe-Site injector valves (lilac color in Figure 16). The first one is an anti-reflux valve, i.e. a mechanical valve, whereby liquids and gases flow in one direction only, preventing process flow from reversing. The Safe-Site valve injects the fluid in a specific direction. These valves have to be located in specific positions in the system, primarily in PBR ports. The precise positions are shown in Figure 22. In some cases, both valves should be put together but in others, only one of them has to be placed. In this last case, the color of the valve to use is also specified in Figure 21. As well as air filters, valves have not been placed. They have to be set for culture experiments once the PBR is sterilized and all the other material autoclaved. Moreover, if a valve is stuck, it should be replaced by another one.

Figure 16. Safe-Site vale on the left and check valve on the right.

- **Silicone tubes.** They are the carriers that allow the movement of liquid and gases over the PBR system. There are four types of silicone tubes depending on their diameter and use. They can be seen in Figure 17. According to the sequential numbering of the right-hand figure: (1) 06/08 tube (6 mm of inner diameter and 8 mm of outer diameter) is used for gas flow, (2) 04/06 tube used for PBR output liquids, (3) 02/04 tube to pump culture medium and (4) 01/03 tube for acid/base solutions. As can be appreciated in this figure, tubes 3 and 4 have a thick wall because they have to be very resistant to pump's friction. If the part of the tube that is in contact with the pump is worn, it should be covered with masking tape.

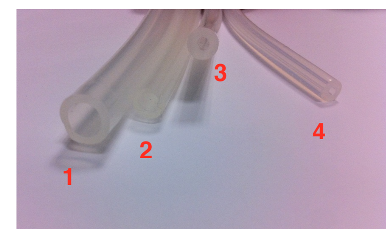


Figure 17. Types of silicone tubes used. (1) 06/08 tube, (2) 04/06 tube, (3) 02/04 tube, (4) 01/03 tube.

With the actual PBR system configuration, there are some tubes that are excessively long, but they have not been cut because the PBR will be moved to another laboratory at some point, and by then, its configuration could change and long silicone tubes might be necessary.

- **Metal pipes.** They are hollow cylinders made of steel. They have a 1/4" diameter and a 5 cm length. There are eight metal pipes in our system, two for each gas device (except for the O_2 sensor). They are placed in the inlet and outlet of each GFC and GFM to propel gas passage and tube clipping, minimizing gas leaks.

Figure 18. Metal pipe.

- **Luer locks.** They are plastic plugs that facilitate silicone tubes's clipping. There are two types: male plugs and female plugs. Male plugs have an external thread while female plugs have an internal thread. The specific position where each type of plug have to be placed is shown in Figure 22.

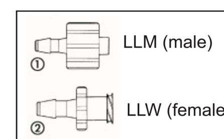


Figure 19. Scheme of both types of plugs.

- **Clamp.** It is used to block liquid passage through a silicone tube. It is placed in the PBR drainage spot to avoid the emptying of it. The clamp should only be removed when an experiment finishes so as to drain the PBR.

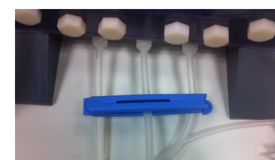


Figure 20. Clamp in port 8.

- **"Y" connector.** It is a plastic piece with a "Y" shape that is used to mix or split gases or liquids that move through the silicone tubes.

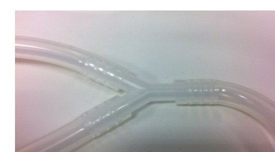


Figure 21. "Y" connector.

3. 2. Assembly of the photobioreactor system

The parts described above were assembled as it is shown in Figure 22. Assembly process was done in a set of steps. Firstly, electric connections were made and after, the fluid piping system. The electric channels are symbolized as black conduits in Figure 22, while the others are represented using a color code.

Concerning electric connections, primarily all the devices with a direct connection to the Control Box were plugged into it following the circuit diagram that can be found in KSD PBR Manual. GFMs and GFCs were connected using usual 9-pol cables. CO_2 GFM connections are not displayed in KSD PBR Manual. In this case, red and orange cables were plugged into pin 62 (+) and pin 28 (-) respectively in PCI-1710UL Multifunction card. LED panels were connected to the transmitter 1G2 shown in Figure 23, red cables to pin 5 and black cables to pin 6. The pH meter has a shielded cable composed of a set of color-coded wires. The connection of each wire to the pH transmitter MV 3010 (see also Figure 23) is the following: A2-black, A4-red, A5-green, A7-white and A8-yellow. Pins A1 to A4 are related with the pH sensor while pins A5 to A8 are matched up with the integrated temperature meter. These six devices are powered and controlled by the Control Box. Likewise, both PCI cards of the Control Box were plugged directly into the computer using two 68-pol cables, so these six electronic devices can be controlled using the Labview program. The Control Box was represented in Figure 22 with a colored box which has a PIC controller (Programmable Interface Controller) inside. This controller is an electronic circuit that can be programmed to carry out a vast range of tasks.

However, the O_2 sensor, the thermal circulator and the air compressor were not connected to the Control Box. They are powered directly by the power supply. The O_2 sensor was also connected to the computer by a serial USB cable. So it can be controlled by Labview and BacVisSingle too. Nevertheless, the termal circulator and the air compressor have an autonomous operation, which means that they cannot be driven by any computer program. They only can be regulated by hand.

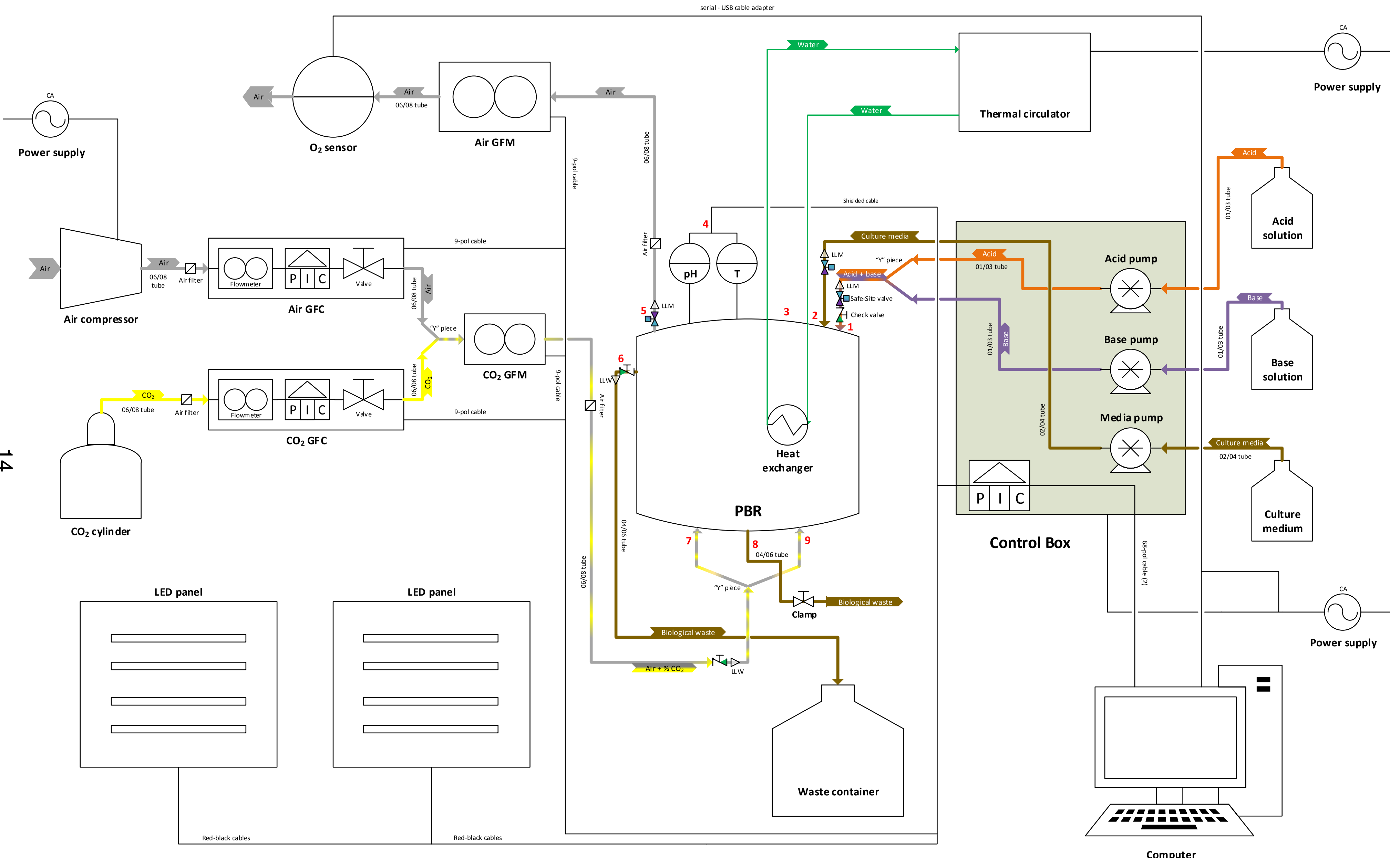


Figure 22. Diagram of the PBR system that sums up the assembly process. It shows electrical connections (black channels), fluid piping system connections (color-coded conduits according to Spanish Regulation UNE 1063 (2000)) between the devices and where the accessory stuff has to be placed. Thick lines represent the main pipes needed for the biological process takes place, and thin lines are referred to secondary connections.

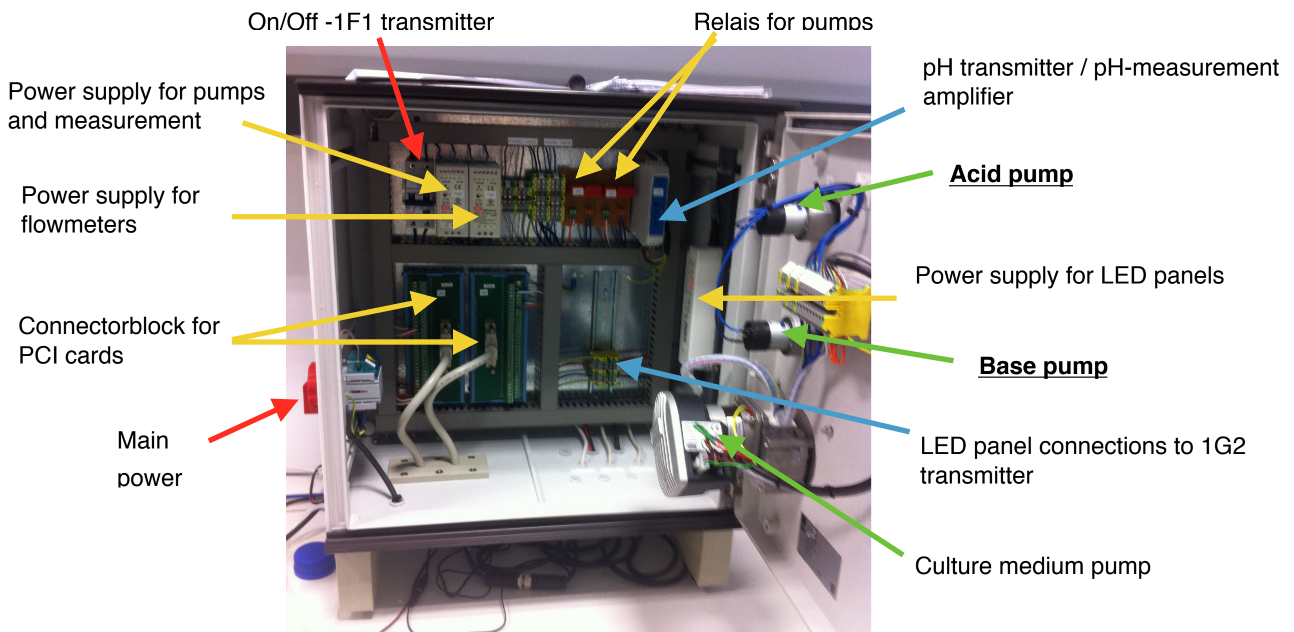
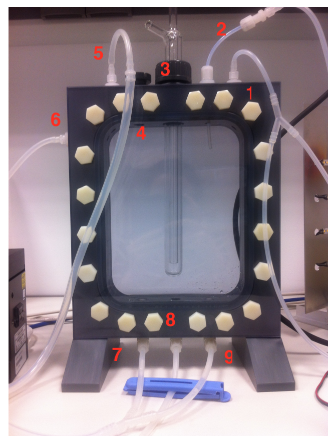


Figure 23. The inside of the Control Box with its components, transmitters and PCI cards

For fluid connections, accessory elements were placed firstly. Male plugs were screwed in ports 1, 2 and 5 and a female plug was squeezed in port 6 (see Figure 24). Metal pipes were set in the gas inlet and outlet spots of each GFC and GFM as it is specified in Figure 25(B). The end pipe on the far side of the widening was put into the screwed plug. The widening avoids gas leaks around the tube. It is important to remember that when the valves are placed, the male and female plugs have to be removed. The valves will be connected directly to their ports according to specifications in Figure 22 and the plugs will be added at the top of valves as it is shown in Figure 25(F).



1. Acid/base input.
2. Culture medium input and initial inocule.
3. Heat exchanger.
4. pH and temperature sensor.
5. Gas output.
6. Liquid output / Biological waste.
7. Gas input.
8. Drainage spot.
9. Gas input.

Figure 24. Flat panel photobioreactor with the nine numbered ports and their usability.

Regarding the silicone tubes, there are four types of silicone tubes as it was commented in section 3.1. In the assembly process, liquid tubes were placed firstly. One of the ends of the acid tube, the base tube and the culture medium tube were placed in their respective Schott-Bottle. The other end of the acid and base tubes were clipped to a “Y” connector and the end of the culture medium tube was clipped to the port 2 male plug (see Figure 22). Besides, an intermediate region of each of these tubes was hooked to its respective pump (see Figure 25(C)) and a small piece of acid/base tube was placed from the “Y” connector to the male plug in port 1. Hence, acid and base solutions enter the same port.

The biological waste overflows by port 6, so a piece of 04/06 silicone tube was place from the PBR port 6 to the waste container. A small piece of this type of tube was also clipped in port 8. As can be appreciated in Figure 24, this last tube is clamped and its bottom-end is not connected to anything. For PBR drainage, remove this clamp and place a container at the tube end to collect the biological culture.

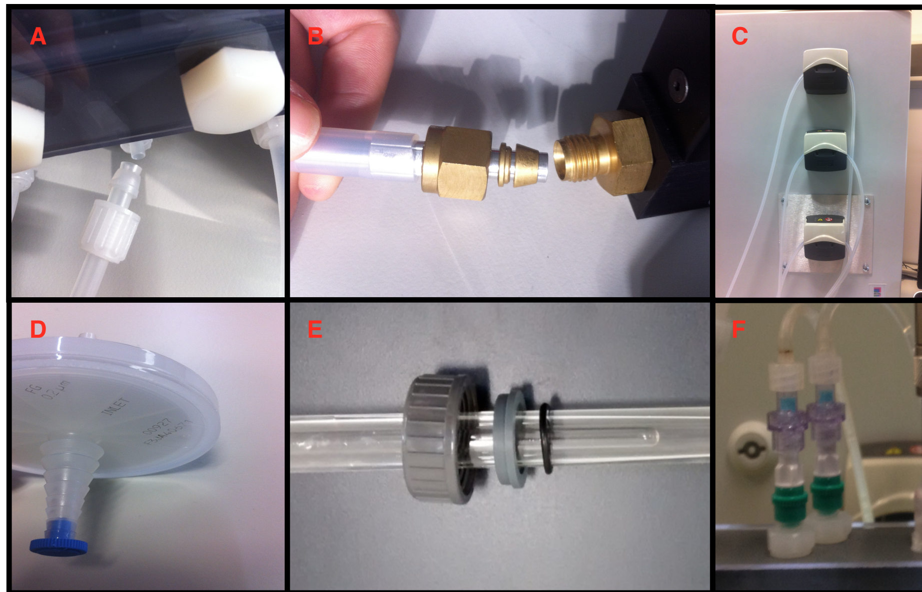


Figure 25. Pictures that show the way in which some components have to be assembled. (A) Clipping of silicone tubes to ports 7, 8 and 9; (B) metal pipe placed at a GFM inlet; (C) silicone tubes hooked to their respective pumps; (D) Air filter inlet side; (E) heat exchanger insertion at port 3; (F) both valves put together with a male plug at the top.

Concerning gases, the gas enters the PBR by ports 7 and 9 and goes out by port 5. Gas outlet is air with a higher O_2 concentration because of the photosynthesis process. Gas inlet is air with an extra percentage of CO_2. Besides, gas inlet generates a bubble column into the PBR that disperses the gas in the culture and causes liquid turbulence for the mixing. The air compressor releases air at a certain pressure and the same happens with the CO_2 bottle. Both streams have to be regulated to a specific flow rate according to the culture needs. Thus, a piece of 06/08 silicone tube was used to connect the air compressor with the Air GFC and the same for the CO_2 bottle and the CO_2 GFC. Outlet GFC gas flows are mixed together using a “Y” connector. 06/08 silicone tubes were placed since the GFC outputs to the connector and from this one to the CO_2 GFM. Hence, this last device measures the amount of CO_2 in the mixed flow that enters in the PBR. This is important to optimize the amount of CO_2 cyanobacteria need to grow. Finally, the 06/08 tube was placed since the CO_2 GFM to the PBR ports 7 and 9 (clipping silicone tubes to ports 7, 8 and 9 as it is shown in Figure 25(A)). Another “Y” connector is used to split the mixed flow. Besides, in this last tube section, an air filter and a Safe-Site valve must be placed (see Figure 22). The air filter has to be set near the GFM outlet to keep it dry. This is due to the fact that a small volume of PBR culture solution always moves out by ports 7 and 9 towards the CO_2 GFM when the PBR is filled. For this very reason, this last tube section is attached to a coupler to keep it in a vertical position.

Furthermore, two more 06/08 tube pieces are placed from PBR port 5 to Air GFM and from this one to the O_2 sensor. The outgoing O_2 sensor filtered gas is released to the environment. This device combination allows the measurement of the % Vol. of O_2 that is in the total amount of PBR out-gas. This measurement gives a lot of information of the culture process like, for example, which the photosynthesis

rate is, whether CO_2 absorption and fixation are taking place or whether photo-oxidation events are happening.

Regarding ports 3 and 4, the pH meter has to be screwed in port 4 when it is going to be used. But when an experiment finishes, it should be unscrewed, cleaned and covered with the cap. The heat exchanger has to be set in port 3 as it is shown in Figure 25(E). As currently we do not have the thermal circulator, the heat exchanger is not operational and it has not been placed. Moreover, neither have we the tubes for water transport that connect these two devices.

3. 3. Computer programs' performance

As it was said before, there are three computer programs for devices control: BacVisSingle, DinModule and LabView. These three programs can be found in the computer desktop.

3.3.1. BacVisSingle

BacVisSingle is used for configuration and zero adjustment of the O_2 sensor. Its user graphic interface is framed using black lines in Figure 26. The sensor is connected to the computer by an USB cable at COM-Port 5. Hence, click the checkbox 5 in *COM-Port* once the program is opened. The *Measuring Interval* is referred to how often the sensor will take measurements which will be visualized in the graphic representation and saved in the data file. The time interval is between 20 seconds and 120 minutes and it can be changed clicking on the arrows. Besides, *Zero Adjustment* checkbox is for one-point-calibration and its checkbox should be clicked for a new measurement but not for a normal measurement in which the same gas is being used. In *Zero Adjustment* the sensor makes a 30 min calibration process which requires exposition to ambient air or nitrogen. If *Zero Adjustment* is not selected, the sensor continues with the measurement according to the last measurement. Finally, in the field *Comments to task* additional information can be written. There, the first row is for the title of the measurement and in the second row, additional data, such as conditions, could be noted.

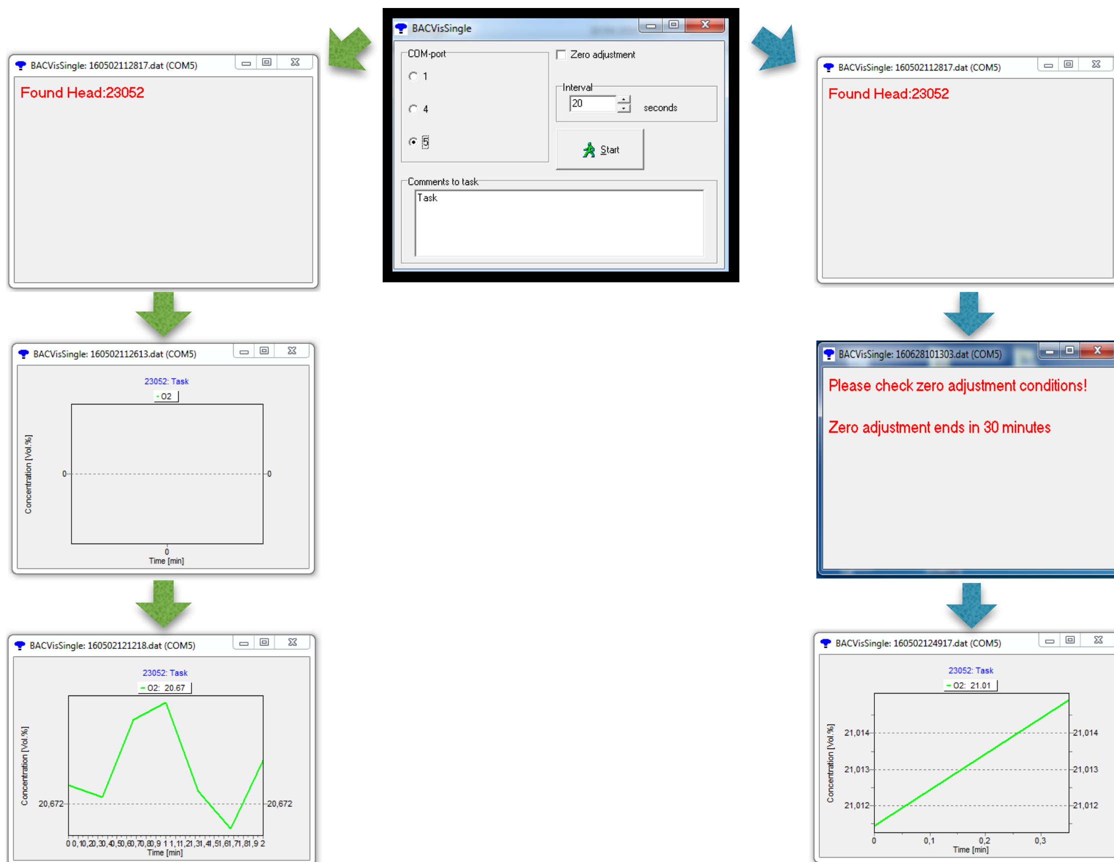


Figure 26. BacVisSingle program. Window with a black frame shows the user graphic interface of the program. The green pathway on the right represents the subsequent windows that will display if Zero Adjustment is not checked. The blue pathway on the left represent the steps if Zero Adjustment is checked.

Once the configuration has been selected (with *Zero Adjustment* unchecked), the button *Start* can be pressed. At that moment, the measuring data will be saved in the directory *.../data* and a new window pops up with a message that indicates the program found a sensor at the selected COM-port. After a short time, a new window with a graphic representation will appear and the measurement will start.

However, if *Zero Adjustment* is checked, a window with the autocalibration remaining time will be displayed. Once the calibration process has finished, a graphic representation will be shown. Moreover, the *Zero Adjustment* is used to minimize the sensor drift. Thus, it has to be done once a month, in a well ventilated room and with normal air (20.97 Vol.% O_2 and 0.04 Vol.% CO_2).

Other information about the program is that relevant data can be copied from the data file even if the measurement is running. It will not stop. Also, it is possible to enlarge a desired area of the curve in the graphic representation by pressing the left mouse button and moving the mouse.

3.3.2. DinModule

For pH meter adjustment, DinModule program should be used. This program allows to take pH and temperature measurements as well as to calibrate and configure the device. Its user graphic interface is shown in Figure 27. This program needs a direct connection between the pH transmitter and the computer using an USB cable with a Phone connector.

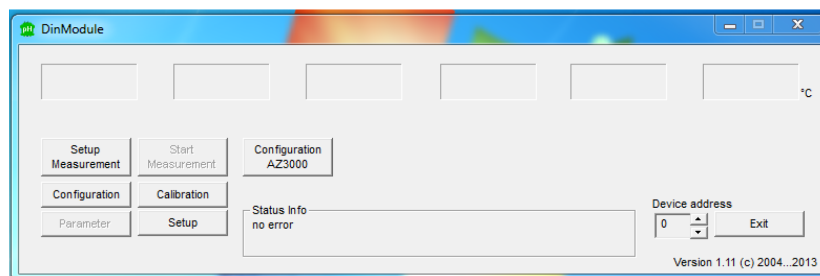


Figure 27. DinModule user graphic interface.

Once the program is opened, *Setup* has to be pressed firstly. In this case, the pH meter COM-port is 4 and the most convenient language has to be selected. Secondly, the correct *Device address* for communication between the transmitter and the PC is 0. About *Configuration* button, it displays the actual configuration data of the measuring transmitter (see Figure 28). In fact, Figure 28 shows the proper configuration parameter values for PBR order, so by default, no data must be changed. There, DAC0 and DAC1 channels are the two analogue outputs and refer to measured parameters. According to our model of transmitter, these parameters are pH and temperature and they have limit ranges between 0-14 for pH and 0-100 ºC for temperature. For this configuration it is also important to define the kind of output parameter (current or voltage) and if current is selected, its range has to be defined (current type 0...20 mA or 4...20 mA). Relay output temperature signal with its limits, the hysteresis and the kind of contact (minimum or maximum) were configured. *Asymmetrie* and *slope* are calibration values that are redefined after each calibration procedure. Calibration key1 and key2 refer to the pH of the standard buffer solutions which will be used in the calibration procedure. Finally, in *Basic adjustments* the adequate temperature value has

to be inserted. Moreover, this value can be fixed if *Fix temperature* checkbox is clicked. *Temperature offset* refers to a reference value that can be used to adjust a linear change of the measured temperature. If the measured temperature is identical to the reference temperature, a higher accuracy will be achieved.

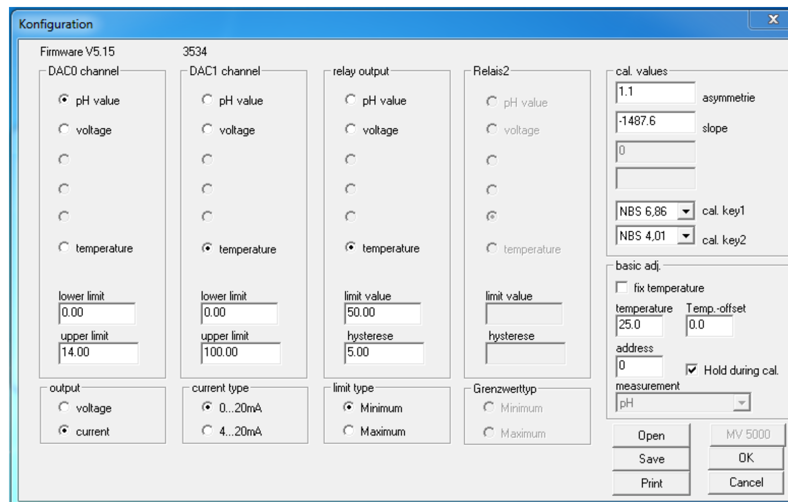


Figure 28. DinModule Configuration.

After parameters have been set, measurements can be taken and calibration procedure can also be done. If *Setup Measurement* is pressed, the program will ask for the datafile name in which the measured values will be save. After, another pop-up window with the *Recording Interval* will display. It refers to the scan rate and establishes how often the measured data is saved in the file. Once the configuration is set up, *Start Measurement* will be activated and the evaluation will start after pressing this button.

However, if the *Calibration* button is pressed, calibration procedure will start. According to the calibration points specified in Configuration, if the feature of the stored temperature relation of the standards is used, it is necessary to select applicable standards from defined sets (pH Buffer Solution acc. NBS) by selecting the nominal values at 25 °C. In addition, it is possible to select and define individual standards but then, the temperature relation cannot be recognized. Thus, after pressing the button *Calibration*, program steps will be followed. Once the calibration process has finished successfully, a pop-up window appears with the new calculated characteristic sensor data (calibration data) and it should be decided whether the new calibration data will be saved in Configuration or not.

3.3.3. Labview

Lastly, Labview is the main program. It is designed to control all the devices, measure the photobiological process cultivation parameters and save the obtained values in a data file. Its user graphic interface is shown in Figure 29 and according to the numbering of this figure: (1) it is a window that shows warnings and comments about the program configuration and allows to save comments with associated timestamps; (2) it gives some information about the program and the data saved; (3) it is the *Welcome card* and it has the *Before you start!* button where some extra information about the O_2 sensor, the set-up, Advantech PCI cards and Run Labview is provided; (4) it is the *Settings register card* that is divided into nine sub-register cards in which the process parameter values are set; (5) it is the *Graphic card* where the

parameter value evolution during the bioprocess can be visualized; (6) it has four buttons: *Start* makes Labview run once all parameters have been set, *Pause* stops program running which can continue if *Pause* is unclicked, *Stop* finishes the running but the program remains opened and *End* finishes program running and besides Labview is closed; (7) it is the general operation unit and provides an overview of the most important measured values. In addition, once *Stop* or *End* are clicked, the program takes a while to carry out the action. If *Stop* is clicked, the parameter values will have to be set again. The program must be closed always by pressing *End* button, do not close it by pressing *File —> Exit*, since it will not end at all. Besides, BacVisSingle and DinModule cannot be opened and used while Labview is running, this will lead to an error and Labview will close.

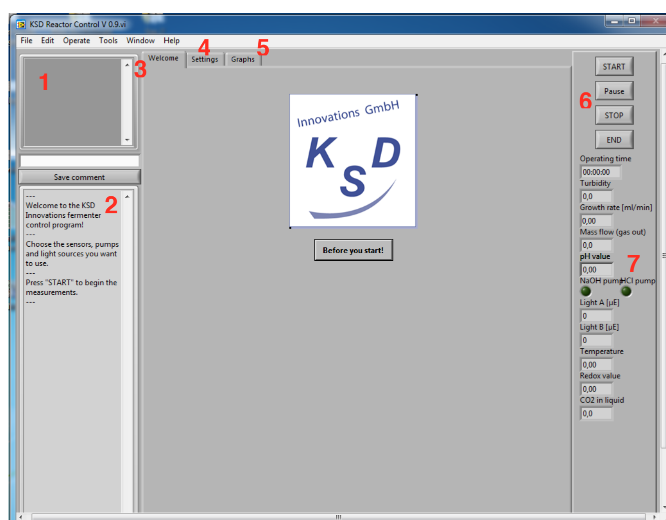


Figure 29. Labview user graphic interface. (1) Warning window; (2) program information window; (3) Welcome card; (4) Setting register card; (5) Graphic register card; (6) Control buttons; (7) General operation unit.

If *Settings card* is clicked, there are nine sub-register cards to set the parameter values of Labview controlled devices. Figure 30 shows the nine cards with the data that has to be inserted. The first card is *Media Flow Rate and Turbidity* for the medium pump configuration (Figure 30(1)). There, *Media pump* button allows to switch the pump on or off. A *Flow rate set point* value has to be inserted as well as the *Channel media*, the *Slope* and the *Intercept* to adjust the analog output signal to the used pump. The flow rate setpoint refers to the rate at which the medium culture is pumped since the Schott-bottle to PBR port 2. The specified *Channel media* is shown in Table 1. The *Slope* and *Intercept* values and the *Flow rate set point* range values were determined according to the experiment results (see Section 4). Moreover, the pump can be switched on and off while Labview is running clicking *Mode* arrows, not the *Media pump* button. The growth rate of the culture is calculated by: (Time media pump is on)*(Pump rate). The time span can be chosen to calculate the growth rate (average).

The second register card is *Light intensity* (Figure 30(2)). Two types of light can be configured. In our PBR system we only have LED panels, so *Light intensity control (fluorescent lamps)* section should be avoided. Regarding LED panels, *LED panel A and B* buttons have to be clicked to On mode. All A and B Channels have to be filled out with number 0. The operation mode has to be chosen in *Source* and Manually [µE] or [V]/[mA] are recommended. Depending on the mode clicked, both A and B LED panels

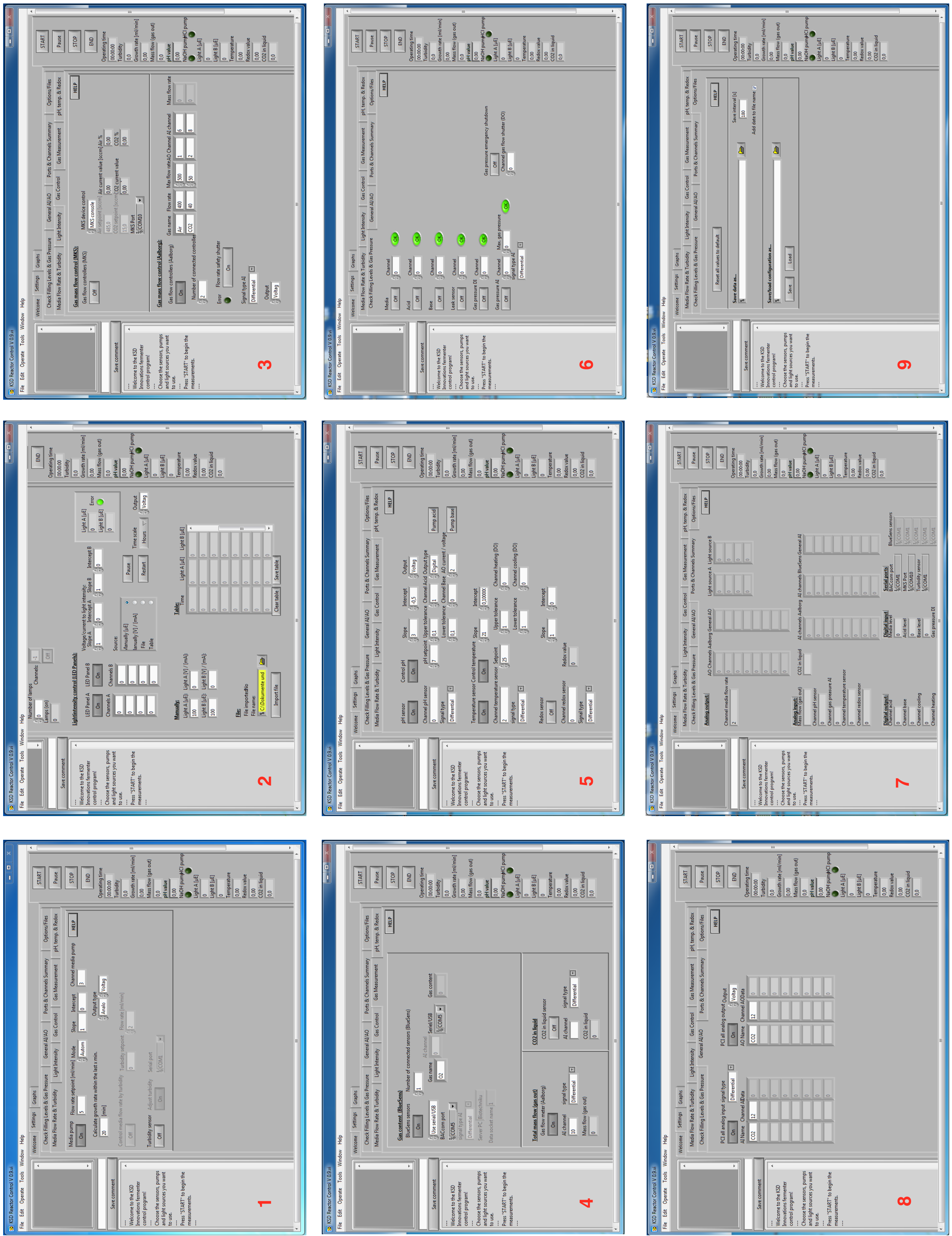


Figure 30. Labview user graphic interface. (1) Media flow rate and turbidity card; (2) Light intensity card; (3) Gas control card; (4) Gas measurement card; (5) pH, temperature and redox card; (6) Check filling levels and gas pressure card; (7) General AI/AO card; (8) Ports and Channels summary card; (9) Options/files card.

boxes have to be filled out in *Manually* section, which refers to radiation intensity ([µE] or [V]/[mA]). If a value of 0µE or 0 [V]/[mA] is set in this last section for both panels, they will be switched off. It does no work for only one panel. Finally, Slope and Intercept values for both panels allow the radiation intensity regulation.

Gas Control card is related to the Gas Flow Controllers (GFCs) (Figure 30(3)). Our devices are Aalborg brand, so only *Gas mass flow control (Aalborg)* section should be configured. Firstly, the *On* button should be pressed. As our PBR system has two GFCs (Air GFC and CO_2 GFC), two connected controllers have to be selected with their respective names, flow rate setpoints, maximal flow rates and channels. The Air GFC has a flow rate range between 0-500 $mL \cdot min^{-1}$ and a maximal rate of 500 $mL \cdot min^{-1}$. However, the CO_2 GFC has a range of 0-50 $mL \cdot min^{-1}$ and hence, a 50 $mL \cdot min^{-1}$ maximal rate. Their respective *AO* and *AI channels* values are in Table 1. They can be switched off, changing the flow rate setpoint to 0. Besides, GFCs may overheat if the gas flow rate is too low while the controller's valves are turned open. *Flow rate safety shutter* is a feature that stops the controllers from overheating and closes the valve if the measured flow rate is smaller than half of the flow rate setpoint. Therefore, the controllers are turned off and eventually they should not be restarted. Also, it is recommended that *Flow rate safety shutter* is turned off for a short time and that the gas flow rate is controlled manually and regularly to ensure the safety of the controllers.

	AI	AO	DI	DO
Media pump		3		
LED pannels		0		
Air GFC signal/setpoint	6	1		
CO_2 GFC signal/setpoint	8	2		
Air GFM signal	10			
pH signal	0			
Temperature signal	2			
Pump small lower (BASE)				0
Pump small upper (ACID)				1
CO_2 GFM signal	12			

Table 1. Specific channels values for each device. AI is referred to Analog Input signal, AO to Analog output, DI to Digital input and DO to Digital output.

The next card, *Gas measurement*, is related to O_2 sensor and Air GFM (Figure 30(4)). In the first section, *Gas content (Bluesens)*, the *On* button should be pushed. Only one controller should be connected since we only have one O_2 sensor in the system. Moreover, the type of connection Bluesens sensor has should be specified, since as it was mentioned above, the sensor is connected to the computer by a serial USB cable adapter to COM5. Thus, serial/USB port and COM5 should be chosen by clicking in the arrows. Next, in Total mass flow (gas out) section the Air GFM configuration should be set. The main button should be turned on and the AI channel box should be filled out with its specific value (see Table 1).

Here, the CO_2 GFM configuration cannot be set since the program came with this configured interface. It will be checked in a following card. Finally, *CO_2 in liquid* section should be avoided for our PBR system.

pH, temperature and redox card is used to configure the pH and temperature sensors (Figure 30(5)). The last R*edox sensor* section should not be filled out since no redox sensor is in our system. Regarding the pH meter, it should also be turned on and the analog input *channel* has to be set according to Table 1. *Slope* and *intercept* values were adjusted based on calibration results (see section 4). Moreover, to control the pH automatically, *Control pH* button should be pressed and *pH setpoint*, *upper tolerance* and *lower tolerance* values should be chosen depending on each culture experiment. Upper and lower tolerance are referred to the tolerable pH deviation from pH setpoint without acid/base pumps activation. Thus, these pumps will turn on if the pH value is higher or lower than setpoint plus/minus the tolerance. Furthermore, *acid* and *base channels* have to be filled out based on Table 1 and *output type* must be changed to digital. *Pump acid* and *Pump base* buttons allow pump activation despite the fact that pH value is within the proper range. Both pumps can be turned off unclicking *Control pH* button, even if Labview is running. Concerning *Temperature sensor* section, the same steps as pH meter should be performed.

Nevertheless, *Check filling levels and gas pressure* and P*orts and channels summary* cards (Figures 30(6) and 30(8)) should not be filled out with any configuration data and in *General AI/AO* card (Figure 30(7)), only CO_2 GFM information has to be set. In this card, both *PCI all analog input* and *PCI all analog output* sections have to be turned on and only their first row will be completed with CO_2 GFM name and its AI/AO channel value displayed in Table 1. Lastly, in *Options/files* card (Figure 30(9)), the above configuration could be saved in a new file or conversely, a configuration file could be loaded instead of configuring all card parameters. *Save data as…* is to create a new file in which all photobiological process data will be saved once Labview is running. Other option is to save the data in a file that already exists by checking the *Add data to file name* checkbox. The time interval at which the data is saved, could also be chosen. Once all parameters were set and files were saved, the Start button can be pressed and Labview will begin running. While the program is running, all the above mentioned parameter values can be regulated and changed to other adequate values.

The signal channels shown in Table 1 are referred to the pin connection each device has with the Control Box. Hence, this values are important for the communication and activation of PBR system devices. In most of the cards there are three repeated features, *Signal type* (Differential, Single-Ended), *Output* (Voltage, Current) and *Output type* (Analog and Digital). These properties must not be changed because they specified the type of electrical connection between the devices and the Control Box .

Finally, in the *Graphic register card* there are four graphics that could give an overview of the raw parameter data evolution during the photobiological process. The graphic time scale can be changed by clicking in the arrows. Each graphic can register until eight different plots of different parameters. Graphic data and graphic representation can be saved by clicking on the right mouse button. The graphic representation is saved by clicking on Export Simplified Image, while the data can be exported to an Excel document or copied to Clipboard and pasted in a text file (i.e. Microsoft Word).

3. 4. Device calibration procedure

Once the PBR is assembled and the computer programs performance is known, the next step is the calibration of some devices that require it. These devices are both GFCs, both GFMs, the O_2 sensor, the pH meter and LED panels.

3.4.1. Gas Flow Controllers (GFCs) and Gas Flow Meters (GFMs)

GFCs and GFMs could not be calibrated because currently we do not have access to a specific type of equipment that includes a flow calibration standard, a certified high sensitive multimeter (which together have a collective accuracy of ±0.25% or better), a flow regulator installed upstream from the device and a pressure regulated source of dry filtered reference gas. Furthermore, a bottle of a reference gas is need but it could not be bought so far because of funding shortage. Once the calibration material is available, the calibration procedure provided at each device manual should be followed.

3.4.2. O_2 sensor

O_2 sensor calibration corresponds to BacVisSingle Zero Adjustment. To get started with the calibration procedure, the following steps must be taken:

1. Connect the sensor to the power and wait about 45min-1hour until the sensor is warm.
2. Turn on the air flow (room air) and open the BacVisSingle program.
3. Firstly, perform a measurement (no Zero Adjustment) (review section 3.3) and wait until O_2% value is stable (usually 30 min and small changes are tolerable).
4. Once the measurement is ended, start Zero Adjustment, which will take other 30 min.
5. Finally, close BacVisSingle. LabView can now be started.

3.4.3. pH meter

The pH meter can be calibrated in two ways: using the DinModule or the three pH transmitter buttons. In both cases, you will need distilled water and the two pH standard solution whose pH must be matched to the pre-defined standards (see cal. key1 and key 2 in Figure 28). Via DinModule is recommended since there are more calibration options and the configuration can be modified. Here, calibration using DinModule is only detailed, for calibration using pH transmitter buttons see pH meter Manual. If DinModule is used, the following steps must be taken:

1. Connect the pH transmitter to the computer using the USB cable with a Phone connector.
2. Open DinModule program and configure the COM-Port in *Setup* and the *Device Address*.
3. Review Calibration parameters in *Calibration* button. If NBS standards are used, *fix temperature* checkbox must be checked.

4. Press *Calibration* button to start the menu-guided calibration.

5. Rinse the electrode with distilled water and dry it with paper.

6. Immerse the electrode in the first pH standard solution (cal. key1 in Figure 27) and confirm the immersion with *OK* in the first pop-up window.

7. Stir the solution and wait until the pH value is stabilized (pay attention to the necessary response time and temperature equilibria) and then press OK.

8. Enter the pH value of the used standard (at the actual temperature) or select the nominal value of the buffer solution at 25 °C of stored buffer sets.

9. Take the electrode out of the first standard solution, rinse it with distilled water, dry it with paper and immerse it in the second pH standard solution. Confirm it with OK.

10. Repeat the steps 7, 8 and 9 with the second standard solution.

11. Once the calibration procedure finishes, a new dialog window appears with the new calculated characteristic sensor data (calibration data). If *Save* button is pressed, these new data will be saved in *Configuration* information, otherwise if *Exit* is pressed, data will not be saved.

3.4.4. LED panels

Finally, LED panels can also be calibrated. For this procedure, a radiometer is needed. A radiometer is a device that measures the radiant flux (light energy) of electromagnetic radiation. The calibration procedure requieres of 100μE of light intensity and a 2 cm distance between LED panels and the PBR, since this is the proper distance for cultivation process. Both panels have to be calibrated separately. The following steps should be taken for the calibration:

1. Set one LED panel at a 2 cm distance from the PBR.

2. Start Labview, configure 100 μE of light intensity and run it.

3. Place the radiometer sensor over the PBR plastic surface and take the measurements diagonally, from an upper corner to a bottom corner. Measurements have to be taken en each LED line and between the lines.

4. With light measurements and the voltage, the calibration graphic can be plotted with the specific slope and intercept of the linear regression line.

5. Repeat the process for the other LED panel.

3. 5. Characterization experiments

3.5.1. Culture medium pump flow rate characterization

For the media pump flow rate characterization ($mL \cdot min^{-1}$), it was used a full water Schott-bottle, test-tubes (250 and 100 mL) and a stopwatch. The pumped deionized water volume from the Schott-bottle to the test-tube at 2 min and at different flow rate setpoints was measured (0.1, 0.5, 0.8, 1, 2, 3, 4, 5, 10, 20, 50, 100, 200 and 500 $mL \cdot min^{-1}$). Two minutes was an adequate time for pump warming and detecting the right pumped water volume. Moreover, different flow rate setpoints were also detected by changing the Labview slope and intercept of the media pump (see Figure 30(1)).

3.5.2. pH control

For acid and base pumps characterization, it was used a full water Schott-bottle, a test-tube (100 mL) and a stopwatch. In this case, pumped deionized water volume from the Schott-bottle to the test-tube was measured at different times (1, 2, 3, 4 and 5 min), but at the same flow rate setpoint. Remember that these pumps can only be switched on and off, so they only have a specific flow rate revolution.

To characterize pH meter operation and Labview functioning, an experiment in which the pH was changing in a water solution over time, was performed. This experiment allowed to see whether the pH meter measured properly, whether the program could detect pH changes and how it respond to these changes activating acid or base pump, increasing or decreasing the pH value. Besides, the pH tolerance setpoint was also studied in order to see if it had any influence in achieving an specific pH setpoint. Prior to the experiment, the PBR was filled up with deionized water, air bubbling was activated and acid (pH 3) and base (pH 11) solutions were prepared. It was waited until CO2 saturation in water and the pH was measured at zero time, being around 5.73 —although it varied due to CO_2 solubility in water. It was adjusted at pH 6.5, as initial pH value. During the experiment, Labview pH setpoints were changed to 7, 6.5 and 7.5, in that order. The tolerance was also changed and interested values were 0.01, 0.05, 0.1 and 0.2.

3.5.3. Optics experiments. LED panels characterization

For optics experiments, a radiometer was used. This instrument measures light radiation intensity in the electromagnetic spectrum (200-1200 nm). Thus, the radiation intensity emitted by one LED panel was measured at three different LED radiation powers (maximum, medium and minimum), at three panel locations (upper right side, middle central side and bottom left side) and at three different distances from the LED panel (2, 3 and 4 cm). Besides, one specific measurement was also taken in the dark — turning off the artificial light sources and blocking sunlight pass through the window — to see whether these other two light sources were having influence in LED light measured values. In addition, PBR plastic surface transmittance was measured. For this, LED radiation intensity at minimum power, on the middle central side and at 3 cm distance was measured and compared with the previous result without the PBR surface.

3.5.4. Warming process and heat transfer

The last experiment consisted on studying the warming process, specifically, the heat transfer from LED panels to the PBR culture at different LED panels distances (1, 2 and 3 cm). Prior to the experiment, the PBR was filled up with deionized water, air bubbling was activated and the temperature was measured at time zero. Afterwards, temperature measurements were taken each 5 min, during 90 min. Furthermore, in order to maximize the heat transfer and basing on optics experiments' results, the radiation intensity was set at its maximum value and the thermometer was placed in the middle central side of the PBR.

4. Results and Discussion

The aim of this work is the assembly, set-up and operation of our 1L scale-lab flat-bed photobioreactor system. For that, a set of steps were carried out (see Figure 1). All these steps require a biological knowledge of the cyanobacteria, the interdependence of the parameters and its effects on cultivation and on the photobiological process. First, all the parts were characterized in order to know how they operate and with which other parts they have to be connected. Once all features were understood, the assembly process was performed as it can be visualized in Figure 22. Each device position and each connection was selected taking into account the cyanobacteria requirements and their cultivation process. Afterwards, it was checked whether everything was properly connected using the already described computer programs. Corresponding program manuals were read for this purpose and their main characteristics are shown in this work (see section 3.3).

Subsequently, device manuals were also read to know their operational mechanisms and how they should be calibrated. With that information, calibration procedures were performed following the steps described in section 3.4. GFCs and GFMs could not be calibrated due to lack of equipment. However, the rest of devices were correctly calibrated according the specifications described before. It should be remarked that LED panels radiation can only be regulated by changing slope and intercept values in the corresponding software menu, and not directly changing their intensity value in *Manually* section (see Figure 30).

Finally, a set of experiments were performed to characterize the operation ranges of all PBR system components. Moreover, these experiments were also useful to ensure that the assembly process was done correctly and to have a better knowledge on how the computer programs have to be used. The characterization experimental results gave relevant information of how the system should operate during real cultivation process. For that, knowledge about the cyanobacteria cultivation procedure, i.e. the devices operation ranges should be previously known.

6.1. Culture medium pump flow rate characterization

It is known that the maximal reached growth rate of *Synechocystis* sp. PCC6803 is 0.135 h^{-1} (Zavřel et al., 2015). However, in normal conditions, growth rates are lower, 0.02-0.03 h^{-1} (Kwon, Rögner and Rexroth, 2012), since there is not enough light and nutrients supply in typical PBR operation conditions. Part of the light that cyanobacteria receive, specially for the case of cells next to the surface, is dissipated as heat or fluorescence, and so light does not reach cells in the internal side of the PBR volume. But, a light intensity increment will cause photoinhibition phenomenon. Thus, it is very difficult to optimize the light. Moreover, the culture medium is not suitable for high culture densities, so it is very difficult to reach optimum growth rates in a photobioreactor (Lopo et al., 2012; Shuler and Kargi, 2002). Consequently, as growth rates are lower, the culture dilution rate in this type of reactors should also be lower, in order not to wash out the culture. Furthermore, for the maximal growth rate of 0.135 h^{-1}, its respective culture dilution rate can be calculated and it is 2.25 $mL \cdot min^{-1}$. So a 3 $mL \cdot min^{-1}$ dilution rate will be enough for cyanobacteria cultivation at high growth rates.

Therefore, the operational flow rate range of our pump was studied, with a special emphasis in lower rates from 1 to 5 mL·min^{-1}. For this first experiment, we assumed that the culture medium pump was well calibrated, so we worked with a Labview slope value of 1 and an intercept of 0. As it can be appreciated in Figure 30(A), at flow rate values lower than one, the pump does not work. In fact, it does not operate either with decimalized values (data not shown). Moreover, we found that at ranges of interest (1-5 mL·min^{-1}), larger volumes than expected were pumped. This fact indicates that the pump should be adjusted changing slope and intercept values in software entry. In addition, at 5 mL·min^{-1} flow rate, the pump reaches its maximum rate, pumping around 200 mL·min^{-1}. At higher rates, the peristaltic pump moves the same water volume (Figure 31(A)). This indicates that the inner diameter of the silicone tube is crucial for the flow rate. At higher inner diameter of the tube, larger volumes can be pumped. This maximum rate could be used to filling up the PBR with the culture medium in the initial experimental stages.

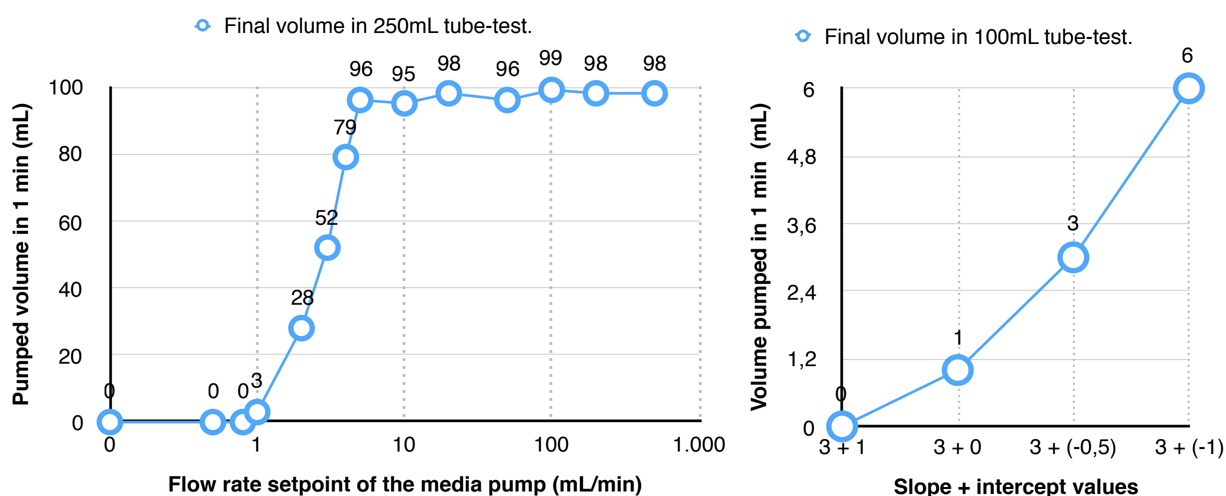


Figure 31. (A) Water volume pumped per min at different flow rate setpoints (with slope 1 and intercept 0) and (B) at different slope and intercept values. Each point represent the resulting volume measured in the test-tube after 1 min.

As the first obtained results were not adequate for our purpose, we tried to calibrate the pump adjusting Labview slope and intercept values. As a 2.25 mL·min^{-1} dilution rate is needed for the maximal growth rate. We wanted to see if a 3 mL·min^{-1} rate is pumped with our pump, since this rate would largely cover high growth rates. Henceforth, we tried to adjust a 3 mL·min^{-1} rate with our pump. To this end, first the intercept was set to zero but the slope value was increased in one unit each time. It was found that at slope 4, the pump does not work and that at slope 2, a volume of 8 mL·min^{-1} was pumped; however, the pumped volume at slope 3 was closer to the objective. Fixing this last slope value and modifying the intercept, it was found that 3 mL·min^{-1} were pumped at slope 3 and intercept (-0,5) (see Figure 31(B)).

Henceforth, a 3 mL·min^{-1} rate can be fixed in our culture medium pump. This rate allows to dilute the culture, set a desirable growth rate by matching dilution rate to growth rate and maintain an exponential growth and stable experimental conditions. Thus, our pump is suitable to largely cover cyanobacteria cultivation.

6.2. pH control

Cyanobacteria, under laboratory conditions, prefer neutral to slightly alkaline culture mediums, with pH values that vary between 7 and 11 (Kratz and Myers, 1955). However, these microbes are also acid-tolerant, since in natural environments, they grow at pH values as low as 4 (Kurian, Phadwal and Mäenpää, 2006). Furthermore, pH control has a fundamental role in minimizing CO_2 losses, due to its influences in mass transfer regulation. Culture yield depends on CO_2 concentration in the culture, so that upper concentrations produces cell inhibition while at concentrations below the minimum limit, cyanobacteria is able to grow. Thus, a good pH modulation is essential to maintain the culture in stable conditions. In addition, carbon dioxide represents a major operational expense of cyanobacteria culture, so pH control is also important to minimize CO_2 losses (Berenguel et al., 2004).

Taking all the above into account, it was considered necessary to perform a pH control characterization experiment. To this aim, first of all acid and base pumps characterization was performed. As it was commented before, both pumps pushing acid and base solutions can only be switched on and off, they do not have a speed range. Thus, it is important to know how much volume is pumped per minute. Both pumps have the same configuration, so it was expected to obtain similar results.

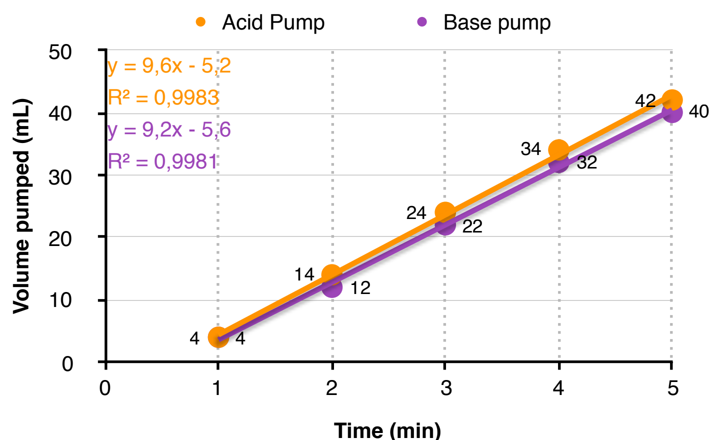


Figure 32. Water volume pumped at different times in a 100 mL test-tube by acid and base pumps. It can be appreciate that flow rate of both pumps is approximately of 10 $mL \cdot min^{-1}$ and that base pumps is a 5% slower than acid pump.

As it is shown in figure 32, in a 5 min range both pumps pumped a pretty identical water volume in a 100 mL test-tube. So, a flow rate of approximately 10 $mL \cdot min^{-1}$ can be detected for both pumps. Around a 5% difference between them was observed, being the base pump a bit slower. This difference indicates that acid pH will stabilize a bit faster than base pH. Furthermore, it can also be seen that during the first minute, both pumps only move 4 mL of water volume. This indicates that both pumps need a warming process, that reduce the pumped volume at the beginning.

Once acid and base pumps were assessed, another pH control characterization experiment was performed. This experiment helped to check if the pH meter correctly works and whether Labview program detects pH changes and can respond to them activating acid or base pump. Prior to the experiment, we realized

that the pH meter had also to be calibrated in Labview program. Using the same pH solution standards as in section 3.4.3., calibration resulted in slope 3 and intercept (-0.5).

During the experiment, we wanted to know how long takes to stabilize pH value when it was changed. Thus, we chose a 6.5-7.5 pH range (neutral region). Then for this interval, pH value varies quickly. Furthermore, four pH tolerance errors were checked (0.01, 0.05, 0.1 and 0.2) to see their influence in reaching the pH setpoint. Figure 33 shows the experimental results.

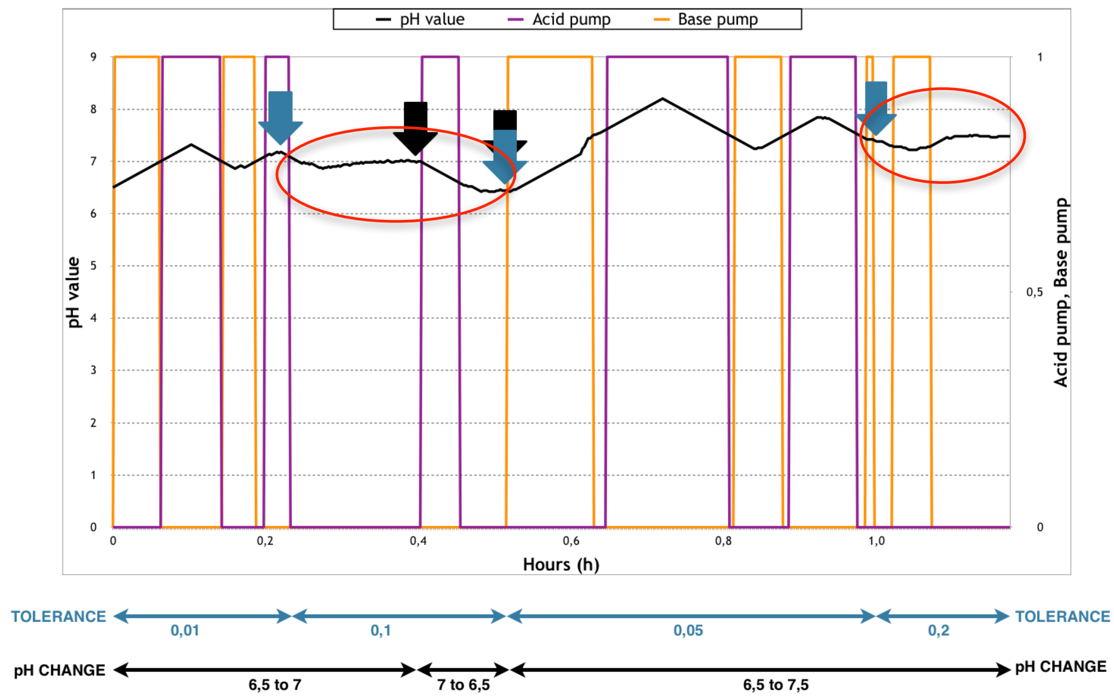


Figure 33. pH evolution during approximately 80 min. A value of 1 in Y pumps axis, means that the pump is on, while 0 value indicate that the pump is off. Moreover, blow the graphic, it is shown at which points pH value and tolerance ranges where changed.

As it can be appreciated, at a tolerance of 0.01 and 0.05, the pH value is not stabilized for the given setpoint or it takes too long. However, at tolerances of 0.1 and 0.2 the pH value stabilizes rapidly (see red circles in Figure 33). This happens because mixing process in our PBR system is done by a pneumatic device (bubbling). This implies that acid and base solution process takes longer, once acid and base pump have been switched off. Hence, at 0.1 and 0.2 of tolerance, a larger time interval is given for the solution process until the pH setpoint is reached. As a tolerance of 0.2 implies a wider range in which the pH can fluctuate, tolerance can be progressively reduced when the pH is closer to the setpoint to reach the desired pH value.

The pH of used acid and base solutions should also be taken into account. The volume of our PBR is relative small, so concentrated acid and base solutions can greatly affect pH in the culture. Hence, for acid and base solutions with extreme pH, higher tolerance values are recommended to avoid input of strong acid and base fluctuating conditions that could damage the cells and affect the culture yield. However, if it is desired to maintain the culture at a specific pH value, for acid and base solutions with less extreme pH, lower tolerance values are recommended. In our case, acid and base solutions with less extreme pH

were used for the experiments, due to lack of safety measures in the laboratory. However, in real conditions, both solutions are usually 0,1M (Kwon, Rögner and Rexroth, 2012), so the effect is almost instantaneous and the dilution rate is not affected.

6.3. Optics experiments. LED panels characterization

Light is one of the central factors to be optimized. It is the fundamental energy source for photosynthesis process and thereby, for cyanobacteria metabolism. These photosynthetic organisms use radiation in the wavelength range of 400-700 nm, the so called PAR radiation. Besides, cyanobacteria can grow under white light intensity range of 100-300 μmol photons $\cdot m^{-2} \cdot s^{-1}$ (Lopo et al., 2012). However, in some cases it can be extended to 400-600 μmol photons $\cdot m^{-2} \cdot s^{-1}$ depending on the strain, time of exposure, cell density and PBR depth (Zavřel et al., 2015). Above these values, usually photoinhibition process starts. Light intensity also influences other variables: an increment in the intensity increases the photosynthetic rate and CO_2 uptake, rising the pH. Thus, the characterization of our artificial light sources (LED panels) was performed to exactly know its provided radiation intensities.

As we said, LED panels were characterized in terms of their wavelength spectrum, their irradiance range and to check whether they provide the same light quantity over all the PBR surface and at different distances from the reactor. For that, a radiometer that measures light intensity in the electromagnetic spectrum (200-1200 nm), was used and measurements were performed by placing the radiometer sensor between the different LED strips. Figure 34 shows one measurement taken by the radiometer.

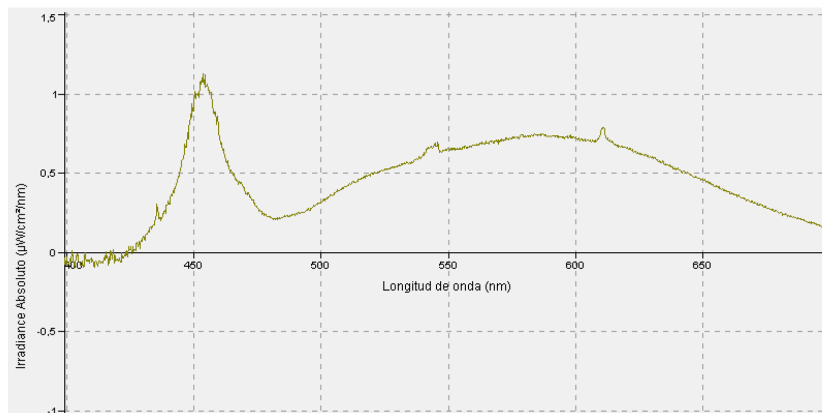


Figure 34. Characteristic wavelength emission spectrum of LED panels light that corresponds to PAR radiation.

In figure 34, it can be seen that the light of the LED panel mainly corresponds to PAR radiation (400-700 nm). A peak near 450 nm can be observed as well as some incident radiation at longer wavelengths, as expected for cool white LED lamps.

For the first experiment, three LED intensity values were chosen: maximum intensity at 717 μE, medium intensity at 211 μE and minimum intensity at 98 μE. This light intensity values correspond to the average of the measurements taken. Considering all measurements taken, a 1138 μE value was obtained, being it near the maximum radiation LEDs can provide. However, the minimum measure taken was of 80 μE, and below this vale, LED panels switch off. Besides, maximum intensity that can be emitted by the LED

panel is a photoinhibition typical value while mid and low intensities chosen are within characteristic *Synechosytis* sp. PCC6803 light intensity cultivation ranges.

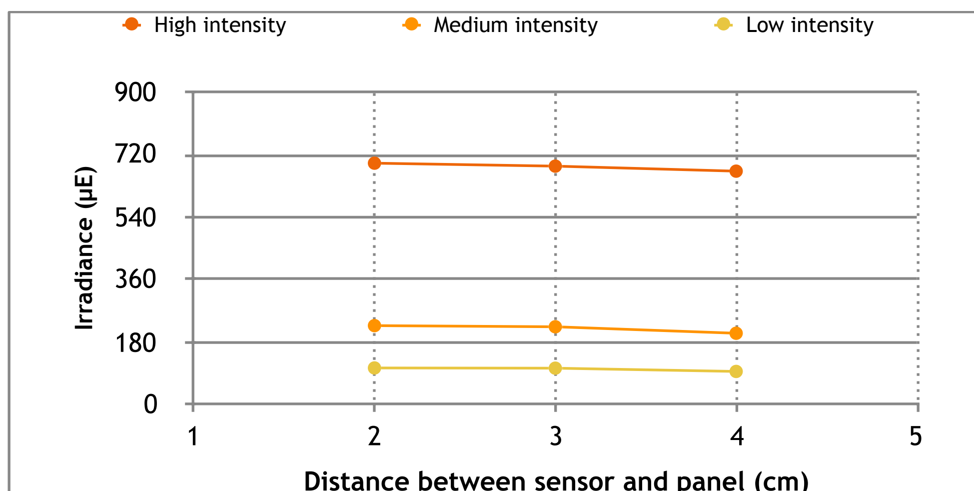


Figure 35. Changes in LED panel irradiance emitted when the radiometer sensor is moved away from the LED panel (at 2, 3 and 4 cm distance). Each dot represent the average of the three measurements taken in the three LED panel positions for each light intensity and distance (see Figure 36).

All the spectral results were integrated in PAR radiation range obtaining a value of irradiance emitted in µE (1µE = 1 µmol photon $\cdot m^{-2} \cdot s^{-1}$). Moreover, three distances between the LED panels position and the PBR were chosen to study how the distance influences the intensity cell receive at the same LED power. Distances chosen were 2, 3 and 4 cm to avoid light losses. Measurements were not taken at 1 cm, since for cyanobacteria cultivation, LED panels are usually placed at least at 2 cm.

Figure 35 shows LED panel light intensity against distance. Each dot represents the average of the three measurements taken in the three LED panels positions for each light intensity and distance (see Figure 36). It can be seen that light energy decreases with the distance. At 2 and 3 cm, irradiance difference is 2%, being the irradiance practically the same for both distances. Thus, placing LED panels at 2 or 3 cm distance should not deliver different results for the cultivation process. However, at 4 cm distance, the irradiance decreases a 10% respect to 2 cm distance. In this case significant differences should be appreciated during cultivation.

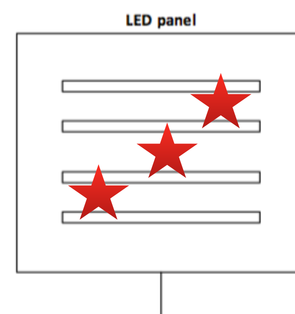


Figure 36. LED panel positions where radiometer measurements were taken.

Moreover, one measurement was also taken in dark conditions to see if environmental light sources could influence the cyanobacteria culture. The measurement was taken at 2 cm, in the center of the panel and at high light intensity (717 µE). The resultant irradiance value was 769 µE. It did not differ so much from the same measurement in light conditions which was 762 µE. Thus, a no significant 1% difference between both values was detected. It was expected that no important differences were detected between light and dark conditions, since LED panels are usually placed at the minimum distance from the PBR. In any case, further measurements in dark conditions with lower intensity values and at different distances should be performed to ensure that there are no significant differences between both conditions.

Finally, the transmittance of the PBR plastic surface was measured to see if this surface absorbs radiation in the PAR range. This fact would decrease radiation received by cells and it should be taken into account for cultivation. Measurements were taken at 3 cm distance, since the instrumentation used does not allow to place the sensor near LED panel when the PBR plastic surface was placed in the middle. Moreover, the measurement was taken in the center of the panel, since it is the region where there will be a higher amount of cells. Any of the aforementioned light intensity values is specifically relevant to estimate the transmittance since the PBR plastic surface always absorbs light in a proportional manner. Hence, the light intensity of 211 μE was chosen. The experimental results show that the total amount of moles of photons transmitted through the PBR plastic surface was 253 μE, while the incident value was 282 μE. Hence, the transmittance could be calculated dividing both values. The result is 90% moles of photons are transmitted through the PBR surface. So, the PBR plastic surface somehow decreases the light intensity transmitted. Moreover, for cyanobacteria cultivation, it is usually assumed that used PBR plastic surfaces do not absorb light. However, here we see that it is not entirely true and that our surface absorb a 10% of the radiation transmitted. This is a substantial reduction to be taken into account in cultivation process.

6.4. Warming process and heat transfer

Cyanobacteria can be found in environments with quite different temperature ranges. However, most of them are mesophilic and have growth optima between 20 and 38ºC (Lopo et al., 2012; Zavřel et al., 2015). *Synechocystis* sp. PCC6803 has an optimum growth between 30 and 33°C under higher irradiance of 500 μmol photon $\cdot m^{-2} \cdot s^{-1}$ but also with lower growth rates (0.01–0.03 h^{-1}). It has been seen that this cyanobacterium adapts to lower temperatures better compared to other microorganisms. Thus, at higher temperatures than 38 ºC some physiological changes happened, such as structural modifications of plasma membranes or oxygen radicals activation among others, decreasing the growth rate. In fact, growth inhibition occurs at 44 ºC (Zavřel et al., 2015).

Artificial light sources have a direct effect in temperature rise. This fact causes that CO_2 and O_2 are less solubles and that the pH increases. Moreover, cell metabolism also produces energy and increases culture temperature. All of this reduce the growth rate and the biomass yield. Hence, a refrigeration system is necessary for cyanobacteria cultivation and it increases energy consumption.

It is known LED panels are usually next to the PBR during cultivation. The emitted light and LED panels warming increases the temperature of the culture. To see, the impact of LED panels in the temperature increment, a last experiment was performed. For that, the temperature was measured with the PBR filled of water and with air-CO_2 saturation during 90 min and at three LED panels distances (1, 2 and 3 cm). Besides, the maximum LED panels radiation intensity was set to maximize heat transfer. The thermometer was placed in the center of the PBR to properly measure. So, the experimental results shown the most extreme case of heat transfer, and without taking into consideration the warming due to cell metabolism.

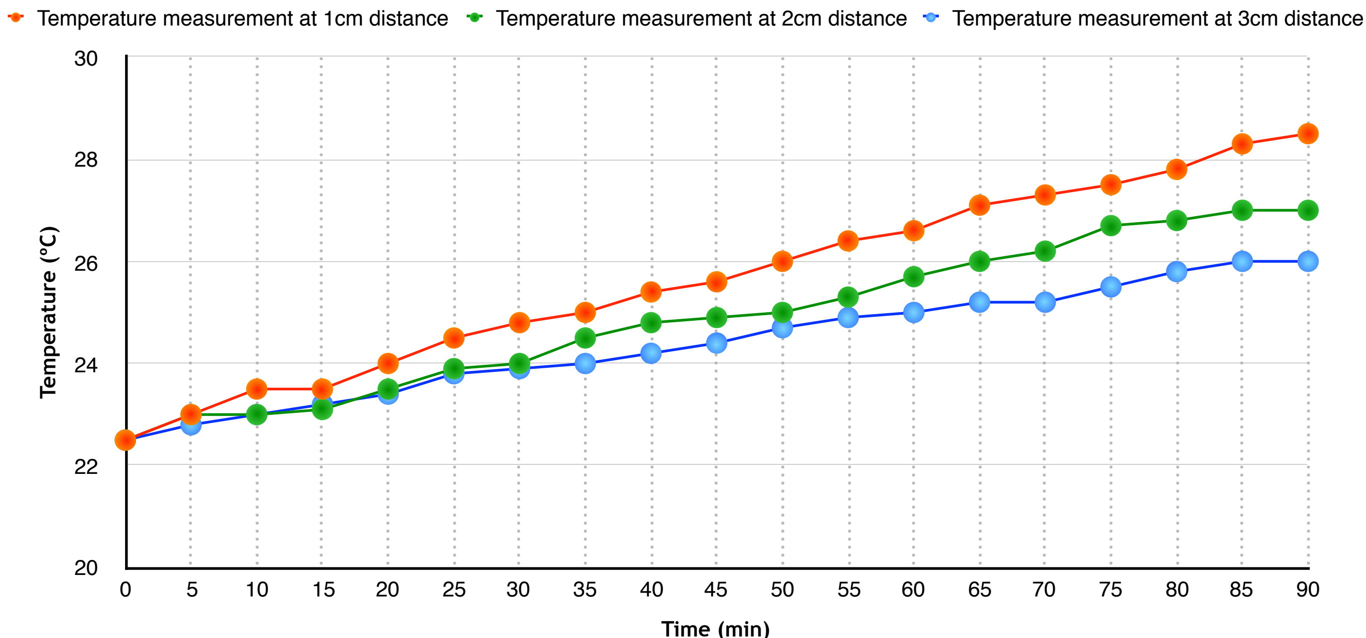


Figure 37. Temperature evolution in a 90 min time interval at three different LED panels distance from the PBR (1, 2 and 3 cm). The experiment was done flanking the PBR with both panel and using maximum LED light intensity.

In Figure 37, it can be shown that at 1 cm distance, the temperature increases more rapidly than at 2 and 3 cm distance. This indicates that the closer LED panels are to the PBR at high light intensities, a better control of temperature should be considered. Moreover, during first 30 min no significant temperature variation was detected between 2 and 3 cm distance. The difference could be appreciated over time. As it was commented before, cyanobacteria have relative low growth rates, so long periods of time for cultivation are necessary. Consequently, a 3 cm distance is recommended over 2 cm distances to avoid overheating of the culture. Besides, irradiance difference between 2 and 3 cm distance is not significant (remembering results shown in Figure 35). Thus, 3 cm distance is still a better option, since at this LED panels distance, light irradiance can be maintained and higher refrigeration costs could be avoided.

5. Future prospects

Some further actions have to be taken if cell cultures are to be grown. Thus, regarding the assembly process, some components have to be placed to carry out culture experiments. Air filters as well as check and Safe-Site valves should be placed in accordance to Figure 21. Moreover, a feed port specific for ISO glass jars should be put in each Schott-bottle stopper (see Figure 38). This piece has two ports, one is used for liquid output and the other one allows air inlet, preventing vacuum generation inside the Schott-Bottle. An air filter has to be placed in the gas inlet to avoid liquid contamination. Moreover, the thermal circulator and the CO_2 cylinder have to be bought and also the 10/12 silicone tubes for water movement between the thermal circulator and the heat exchanger. The waste container should be replaced by a container suitable for biological waste.

Figure 38. Schott-bottle with the feed port specific for ISO glass jars and the air filter.

If it is wanted to carry out more accurate light experiments, some guide-rails can be bought to fix LED panels and control its distance to the PBR. A scaffolding or a block structure for gas devices (GFCs, GFMs and O_2 sensor) could also be built. Hence, gas devices can be fixed to it, being protected from impacts and making its visualization easier. Besides, a gas-liquid phase separator could also be bought. When liquid overflows through port 6, some gas can flow out with it too. So, the phase separator will separate both fluids and the outlet gas could be recirculated and mixed with port 5 outlet gas to get more accurate gas measurements by Air GFM and O_2 sensor. However, these recommendations are not strictly necessary since the PBR can also operate without them.

Furthermore, if the PBR system is moved to another laboratory, the next aspects should be taken into consideration: the laboratory has to be adapted to have a CO_2 cylinder inside and must have specific equipment for the photobiological process. In addition, the air compressor has to be placed in a different bench, apart from the other PBR system components, since the compressor vibrates when it is operating and these vibrations can affect the other devices and even, the photobiological process.

Further characterization experiments could be done before cultivation. The first one is a mass transfer characterization. For that, turbulence should be measured inside the PBR at different gas flow rates. Each gas flow rate produces a specific inlet gas pressure, resulting in a particular bubble size. Lower bubble sizes facilitate mass transfer, since the surface area per volume unit increases and the ascent rate is lower, remaining the bubble more time in contact with the culture. However, higher bubble sizes increase culture turbulence and mixing process but cause greater cell damage which could be detrimental for the bioprocess. Thus, finding a trade-off between the turbulence and the cell damage is important for the culture. The second experiment is about heat transfer characterization. It could be interesting to know the water flow be pumped by the thermal circulator, the water temperature for culture cooling and the time it does take to reduce the culture temperature one degree Celsius.

Furthermore, it would be interesting to carry out a few more optics experiments for LED panels light characterization. It could be studied whether there are significant differences between dark and light conditions, to check at which specific wavelengths the PBR plastic surface absorbs and also verify whether the culture medium BG11 absorbs light due to its mineral composition.

Finally, a sterilization process for most of the PBR components should be performed before cultivation. This is the most important step before cultivation, since if it is not properly done, there will be biological contamination, cyanobacteria will grow suboptimally and the overall photobiological performance will be reduced. A chemical sterilization protocol by incubation with 5mM peroxyacetic acid solution for 1 h can be found in PBR Manual.

6. Conclusions

All the proposed objectives have been achieved and can be summarized by the following results:

1. The assembly and start-up of a 1L lab-scale flat-bed photobioreactor system was performed. For that, all the parts, components and devices were first characterized to know their specifications and operational principles.

2. Thanks to the data collected during the characterization process and taking into account previous biological knowledge in cyanobacteria cultivation and photobiological processes, all components could be interconnected and assembled making up the photobioreactor system.

3. According to software manuals specifications and additional information supplied by the producer company, the correct program functioning could be assessed. Besides, computer programs were used to ensure that the assembly and set-up were performed properly.

4. Calibration procedures were determined and correctly done thanks to the information provided by their respective manuals.

5. A set of characterization experiments were performed, obtaining relevant results that should be taken into account for future cultivation process.

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