Instruction Guide

PHOTON SYSTEMS INSTRUMENTS

Large-scale PBR 25 l / 100 l

Please read the Guide before operating this product

TABLE OF CONTENTS

1 WARNINGS AND SAFETY PRECAUTIONS

GENERAL ELECTRICAL SAFETY GUIDELINES:
- Routinely check the devices and their wiring.
- Replace worn or damaged cords immediately.
- Use electrical extension cords wisely and do not overload them.
- Place the devices on a flat and firm surface. Keep them away from wet floors and counters.
- Avoid touching the device, socket outlet or switch if your hands are wet.
- Do not perform any alterations to the electrical part of the devices or its components.

The following table presents basic highlight symbols used in this manual:

Symbol	Description
⚠️	Important information, read carefully.
ℹ️	Complementary and additional information.

Tab. 1 Used symbols

⚠️	The Photobioreactor PBR 25 l / PBR 100 l is considered Class 1M* LED Product. LED radiation may be harmful to eye. Avoid direct and strongly reflected exposure. Use protective glasses.

*Class 1M: Laser and LED equipment that is safe for the naked eye under foreseeable conditions of operation. Looking directly into the source of radiation by employing optics within the beam, such as magnifying glass, telescope or microscope, can be potentially hazardous.

DANGER — LED RADIATION — USE PROTECTIVE GLASSES — CLASS 1M LED PRODUCT

2 GENERAL DESCRIPTION

The Photobioreactor (PBR) is designed for high-content, precise phototrophic cultivation of algae and cyanobacteria. The system features a unique combination of the cultivation unit and computer-controlled monitoring device.

Cultivation conditions can be dynamically varied according to user defined protocol. Various characteristics including irradiance intensity and timing, temperature and aeration gas can be adjusted with high accuracy. Continuous turbidostatic cultivation can be used to stabilize biomass suspensions by controlling optical density. In addition, chemostat or pH-stat cultivation can be coupled to the photobioreactor for high-precision control of cultivation conditions.

Supplementary sensors for monitoring and control of pH/T, dO_2, dCO_2 are available. List of parameters measured and/or calculated by the Photobioreactor in full configuration is introduced in Tab. 2.

The growth of the cultures is monitored by the integrated densitometer (OD 720, 680 nm). The instantaneous physiological state of the culture is measured by the Photosystem II quantum yield Fv/Fm.

The entire cultivation system is controlled by PBR Control Software, which provides an intuitive and dynamic interface for setting of cultivation parameters, real-time visualization of recorded data and remote access.

The PBR system is designed to be modular so that different configurations can be purchased and manufactured to reflect the user's needs. Basic version allows fundamental large-scale cultivation with defined irradiance whereas fully equipped system provides thoroughly controlled and monitored high-content, precise biomass cultivation.

Symbol	Name	Description
T	Temperature (o C)	
pH	pH	Negative decadal logarithm of hydrogen ion concentration in the medium
O_2	Oxygen concentration	Concentration of dissolved oxygen
CO_2	Carbon dioxide concentration	Concentration of dissolved carbon dioxide
OD_{680}	Optical density at 680 nm	Optical density representing light scattering and chlorophyll absorption
OD_{720}	Optical density at 720 nm	Optical density representing light scattering
OD_{680}/OD_{720}		Relative chlorophyll content of the culture
$OD_{680}-OD_{720}$		
F_t (Dark)	Instantaneous fluorescence in dark	Instantaneous F-yield in dark equals F0 Ft = F0 (in dark-adapted sample)
F_t (Light)	Instantaneous fluorescence in light	Instantaneous F-yield in light that results from a dynamic equilibrium of plastoquinone reducing and re-oxidizing processes and from non-photochemical quenching Ft = Fs (in light-adapted sample)
F_m (Dark)	Maximum fluorescence in dark	Qa reduced, qp = 0
F_m (Light)	Maximum fluorescence in light	
QY (Dark)	Instantaneous PSII quantum yield in dark	Effective PSII quantum yield QY = FV / FM (dark-adapted sample)
QY (Light)	Instantaneous PSII quantum yield in light	Effective PSII quantum yield relaxing in light QY = FV' / FM' (light-adapted sample)

Tab. 2 List of parameters.

2.1 TECHNICAL SPECIFICATIONS

Cultivation Vessel	
Volume	25 or 100 l
Shape	Flat, rectangular
Material	Glass
Lid	Stainless steel, silicone gaskets, gas and electronics fittings
Aeration tube	Plastic U-tube with porous polyethylene sparger (pore size 50 µm)
Sterilization	Chemical sterilization possible

Thermoregulation	
Temperature sensor	Platinum Pt 1000 sensor
Thermoregulation system	1,000 and 2,500 W heater for 25 l and 100 l PBR, respectively
Temperature range	30 - 60 °C with ambient temperature around 20 °C 15 - 60 °C with ambient temperature not exceeding 30 °C (with optional Cooling unit)

Illumination – LED Lighting		
Light panel	Bi-color with separately controllable channels	
Color version	Cool White - Red Other color combination on request	
Total intensity	Up to 500 and 300 µmol. m^{-2}. s^{-1} for White and Red light, respectively	Up to 2,200 µmol. m^{-2}. s^{-1} (with optional Enhanced illumination module) Available for 25 l PBR
Light path	6.5 cm ± 5 %	
Light regime	Light / dark cycles Constant, Linear, Sinusoid light mode Cycles from seconds up to days	Java scripting

Aeration System	
Air sparging	Aeration pump SECOH MKC-510V
Aeration tube	Plastic U-tube with porous polyethylene sparger (pore size 50 µm)
Bubble interruption valve	Bubbling interruption before OD and Chlorophyll-a fluorescence measurement automatically

OD and Chlorophyll-*a* Fluorescence Monitoring (optional)	
Optical module	For OD and Chl-a fluorescence monitoring
Optical density	Real time measurement of OD at 680 and 720 nm
Double-modulation fluorometer	Chl-a fluorescence monitoring induced by blue (455 nm) and orange-red (620 nm) excitation light F_0, F_T, F_M, F'_M, ($F'_M - F_T/F'_M$)
Optical path	10 mm

Sensors (optional)	
Electrode module	Enables connecting up to four measuring sensors to the Photobioreactor
pH module	Digital pH sensor InPro 3253i/SG/325, cable, SW control
dO$_2$ module	Digital optical O$_2$ sensor InPro 6860i/320, cable, SW control
dCO$_2$ module	Digital CO$_2$ sensor InPro 5000i/320, cable, SW control
Accessories (optional)	
Cooling module	For temperature range 15 - 60 °C with ambient temperature not exceeding 30 °C Cooling unit with water chiller Hailea Ultra Ti 1500 HC-500A, stainless-steel water-cooling coil, supporting control software
Gas Mixing System GMS 150	For precise concentration and flow rate of the required gases, input pressure 3 – 5 bar, gas cylinders not included
Turbidostat module	For fully controlled automatic turbidostatic cultivation Two diaphragm pumps and supporting control software
Chemostat module	For fully controlled automatic pH-stat cultivation Two peristaltic pumps + one diaphragm pump and supporting control software
Pumps	Up to 8 peristaltic pumps + up to 2 diaphragm pumps
Control Unit	
Photobioreactor control software	For online monitoring and visualization of all measured data as well as creation of user-defined protocols through a user-friendly graphical interface
Java scripting	Allows creation of additional user-defined specific protocols
Remote access	Ethernet
Others	
Material	Glass cultivation vessel, stainless steel (lid, cooling spiral), aluminum frame, silicone gaskets, plastic fluorometer, polyethylene sparger
Dimension (l × d × h)	25 l cultivator: ca. 103 x 70 x 120 cm 100 l cultivator: ca. 202 x 80 x 160 cm Cooling unit: ca 56 x 68 x 144 cm Control unit: ca. 66 x 64 x 202 cm
Weight	25 l cultivator: 100 kg 100 l cultivator: 300 kg Control unit: 150 kg
Power consumption	25 l cultivator + standard illumination: 450 W 25 l cultivator + Enhanced illumination: 850 W 100 l cultivator: 900 W Heating 25 l: 1,000 W Heating 100 l: 2,500 W Cooling unit: 500 W Control unit: 100 W
Electrical	90 – 240 VAC

2.2 Versions and Configurations

The standard basic version of the photobioreactor consists of Photobioreactor base, Illumination module and Control unit. Different versions and configurations can be selected to optimize the cultivation setup for specific needs.

2.2.1 Versions of Large-Scale PBR

Different versions of large-scale PBRs are characterized by their volume, illumination spectra, and illumination intensity. Once a specific PBR version is chosen, following parameters of the cultivation system can't be changed: cultivation volume of the cultivation cuvette, spectra of the cultivation LED panel, maximal illumination intensity.

Volume	Light Version	Irradiance ($\mu mol. \ m^{-2}. \ s^{-1}$)	Temperature range (ambient temperature around 20 °C)
25 l	Cool White - Red	500 + 300	30 - 60 °C
	Cool White - Red: Enhanced illumination module	1500 + 700	30 - 60 °C
	Customized		30 - 60 °C
100 l	Cool White - Red	500 + 300	30 - 60 °C
	Customized		30 - 60 °C

Tab. 3 Large-scale PBR: Versions

Illustrative spectral characteristics of the standard bi-color LED panel are shown in the Fig. 1.
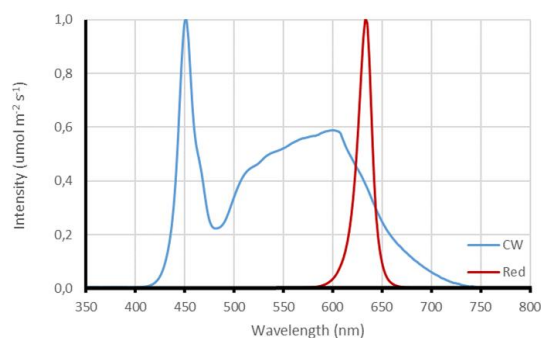
Fig. 1 Illustrative LED spectra of large-scale PBR.

2.2.2 Configuration of Large-Scale PBR

The large-scale PBR cultivation system is modular and can be purchased in different configurations depending on the user's needs. The basic configuration must consist of following modules:

- PBR base: Cultivation vessel (for 25 or 100 l PBR) mounted in a frame equipped with wheels.
- Illumination module (for 25 or 100 l PBR) inbuilt in PBR base.
- Control unit comprising power controller, Photobioreactor controller, light controller, ethernet switch, control computer with Photobioreactor software.

The optional modules extend the use and application of PBR and can be added to the cultivation system subsequently if required. These modules include: Optical module (densitometer with fluorometer), Electrode modules (pH/T, O_2, CO_2), Fluidic modules (Turbidostat, Chemostat/pH-stat, Level-stat), Cooling module, Gas modules (Gas Mixing System GMS 150/TORA).
Please refer to Tab. 4 for a list of all available modules

Module	Components included in the module	Controller (placed in the storing rack)
PBR base	Cultivation vessel (for 25 or 100 l PBR), Foam trap, Temperature sensor, Water level sensors, Heater, and U-tube aeration system with tubular sparger, air pump and bubble interruption valve. The cultivation vessel is mounted in a frame equipped with wheels.	Photobioreactor
Illumination modules	Standard or Enhanced illumination module for 25 l PBR Standard illumination module for 100 l PBR The illumination module is inbuilt in the PBR base and includes electric circuits and ventilator	Light controller
Optical module	Densitometer + fluorometer, cable, supporting control software	Fluorometer
pH module	pH sensor InPro 3253i/SG/325, cable, supporting control software	Probe controller
O_2 module	Optical O_2 sensor InPro 6860i/325, cable, supporting control software	Probe controller
CO_2 module	CO_2 sensor InPro 5000i/325, cable, supporting control software	Probe controller
Turbidostat module	Two diaphragm pumps (inflow/outflow), supporting control software. Optical module with Fluorometer controller has to be purchased with the Turbidostat module.	Photobioreactor
Chemostat/pH stat module	Two peristaltic pumps PP600 (acid + base), one diaphragm pump (outflow), supporting control software. pH module with Probe controller has to be purchased with the Chemostat/pH stat module.	Photobioreactor
Cooling module	Hailea chiller with storage water tank and appropriate pumps. The entire system has its own stand. Water cooling coil, supporting control software Enables widening the range of temperature for cultivation to 15 – 60 °C (ambient temperature must be around 20 °C).	Photobioreactor
Gas Mixing System	GMS 150/TORA-10L for 25 l PBR GMS 150/TORA-20L for 100 l PBR	Control computer
Control unit with a storing rack	Includes control computer with advanced PBR Control Software for control of the Photobioreactor cultivation system. The rack accommodates also air pump with bubble interruption valve, power controller, controllers for individual modules and ethernet switch. Additional equipment as small pumps for Fluidic modules, GMS 150, storage tanks etc. may be placed in the Control unit.	

Tab. 4 Available modules

3 DEVICE DESCRIPTION

3.1 PBR BASE AND CULTIVATION VESSEL

The Photobioreactor base consists of flat cultivation vessel, base box containing electronics circuitry and frame made from duralumin with eloxal coating.

The cultivation vessel has a capacity of 25 l or 100 l (Fig. 1) and it is made from flameproof glass (thickness 12 mm). Its lid is made of stainless steel (AISI316L) and sealed with a silicone gasket. The lid of the vessel is easily attach- and detachable with several clamps. The lid contains connectors for gas or medium supply tubing, waste medium exhaust or sample collection. The larger round ports can accommodate the optional temperature sensor, pH sensor and dissolved O_2 or CO_2 probes. The large angular ports are intended for level sensor, heater, cooler and optical module with inbuilt densitometer and fluorometer. To prevent over-pressurization of the vessel, a pressure relief valve is built into the lid.

There is a U-tube shaped aeration system placed inside the cultivation vessel to ensure biomass mixing and supply with air or CO_2 mixture. The porous plastic gas sparger at the bottom of vessel enables homogenous biomass sparging through the entire surface of the tube. The sparger is connected to the holders that are fixed with plastic nuts to the lid.

The vessel is placed adjacent to an array of high-power light emitting diodes (LED panel) that generate a highly uniform irradiance flux.

Foam trap collects excess foam from biomass. If the Turbidostat module is purchased the inlet/outlet pumps are attached to the frame on the sides of the cultivation chamber. There's a drain valve at the bottom of the cultivation flask to make emptying easier.

The entire base is equipped with wheels for easy handling. For illustrative description of PBR 25 and 100 l including their lids, please see following Fig. 2, Fig. 3, Fig.4 and Fig. 5.

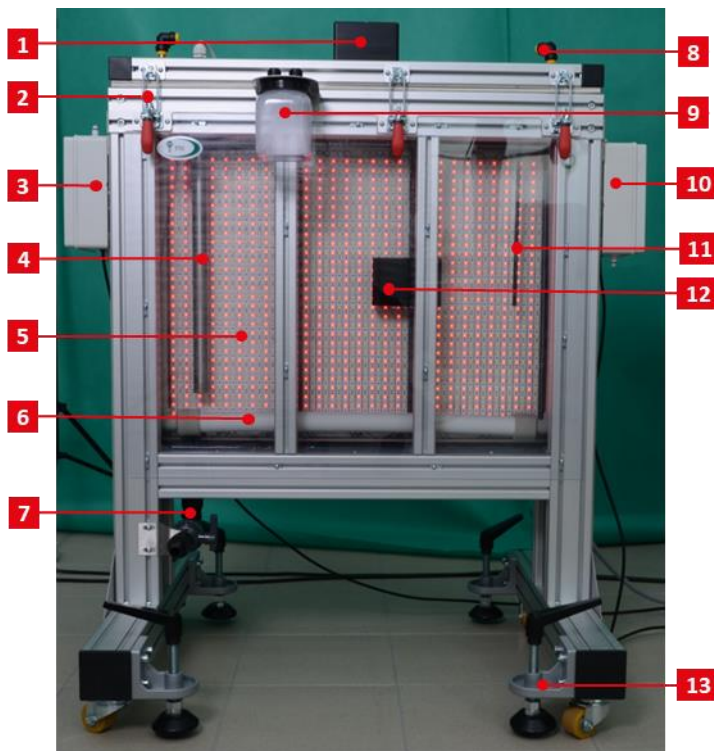|	
(i)	Cultivation vessel is not autoclavable.

Fig. 2 PBR 25 l: vessel description.

(1) Water level sensor. (2) Clips to lock the vessel hood. (3) Turbidostatic input pump. (4) Heater. (5) Light panel. (6) Gas sparging system. (7) Drain valve. (8) Aeration tubing. (9) Foam trap. (10) Turbidostatic output pump (11) Temperature sensor (12) Optical module. (13) Wheels and locking system
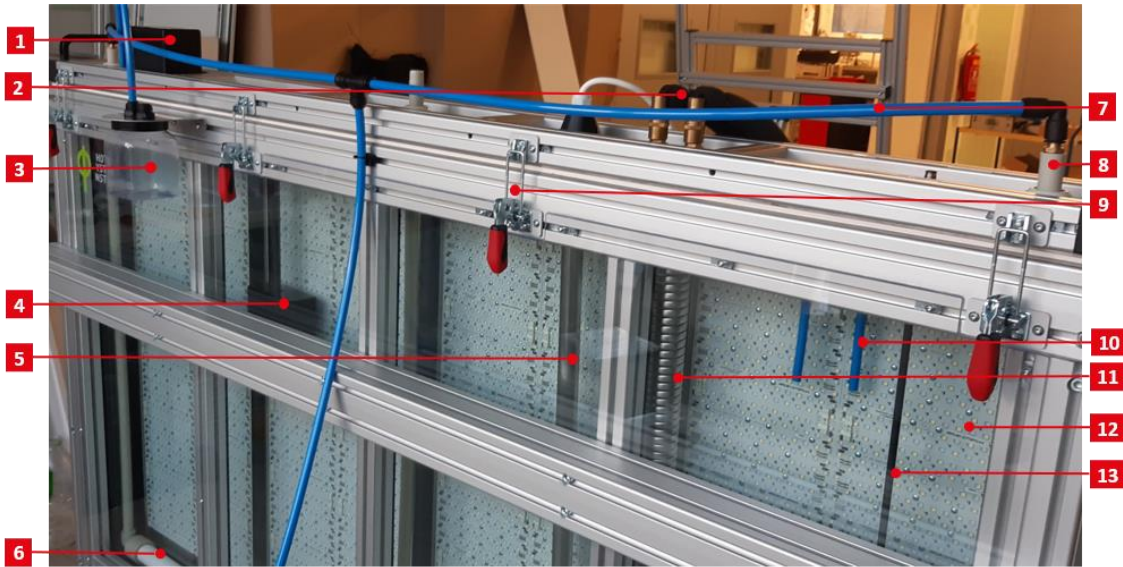
Fig. 3 PBR 100 l: vessel description.

(1) Water level sensor. (2) Cooling houses. (3) Foam trap. (4) Optical module. (5) Heater. (6) Gas sparging system. (7) Aeration tubing. (8) Plastic nuts holding gas sparging system. (9) Clips to lock the vessel hood. (10) Outlet ports. (11) Cooling coil. (12) Light panel. (13) Temperature sensor.
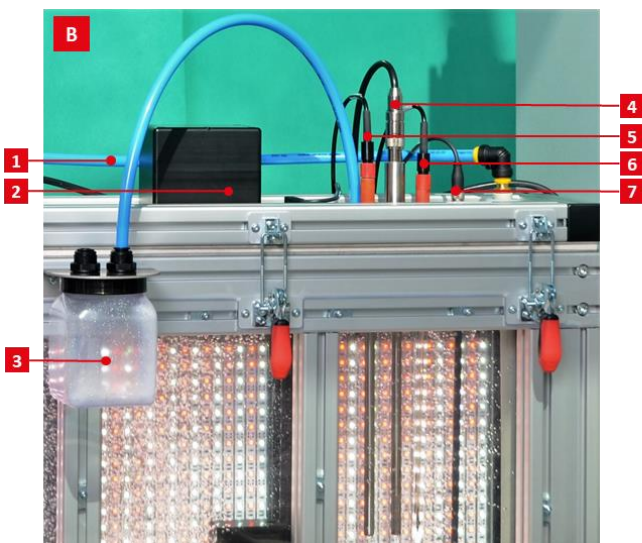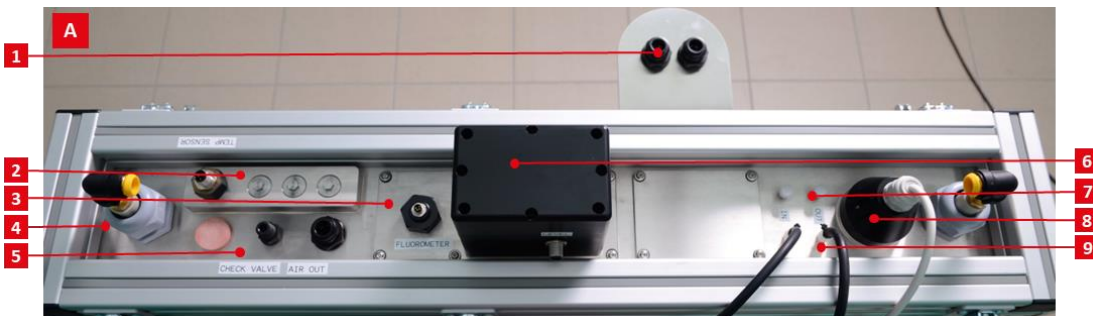

Fig.4 PBR 25 l: lid description.

(A) – (1) Air out port leading to Foam trap. (2) Sensor compartment. (3) Compartment for optical module. (4) Plastic nuts holding gas sparging system. (5) Check valve = pressure relief valve 6) Compartment for water level sensor. (7) Small ports for inlet/outlet tubing (turbidostatic, pH-stat tubing, sampling, etc.). (8) Heater port. (9) Compartment for cooling coil.

(B) – (1) Aeration tubing. (2) Water level sensor. (3) Foam trap. (4) dO_2 sensor. (5) dCO_2 sensor. (6) pH sensor (7) Temperature sensor,

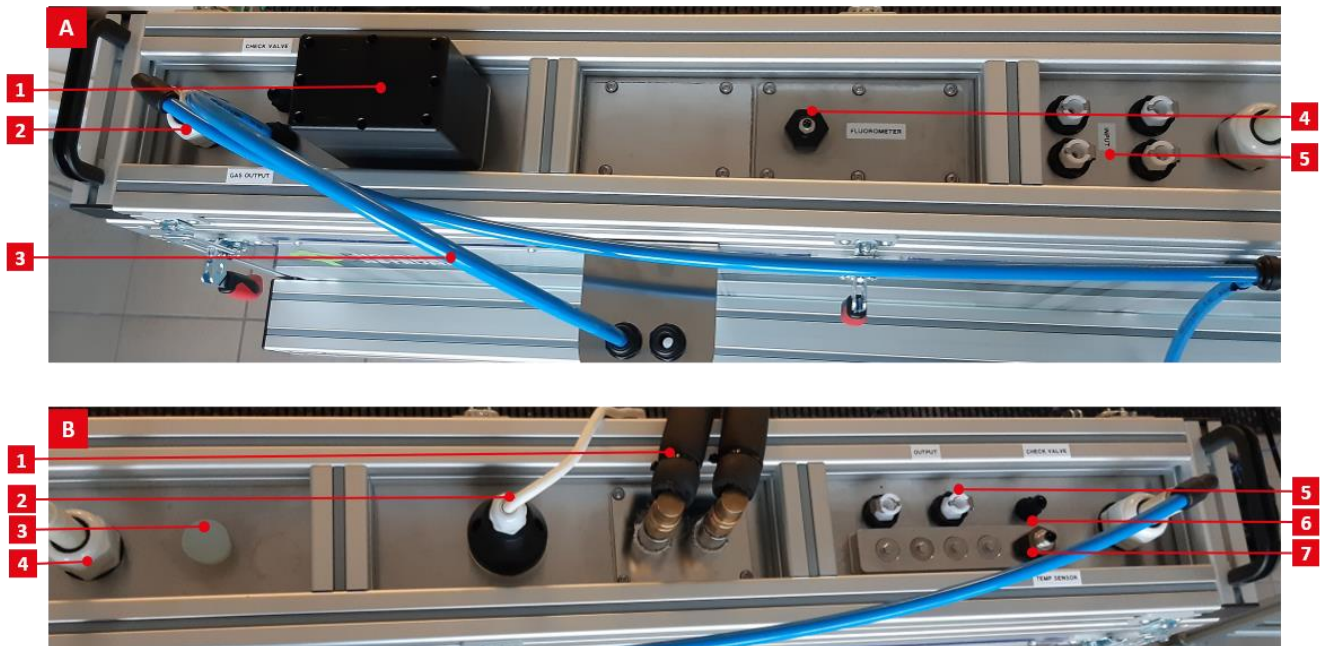**Fig. 5 PBR 100 l: lid description.**

(A) – (1) Water level sensor. (2) Plastic nuts holding gas sparging system. (3) Gas outlet leading to the Foam trap. (4) Compartment for optical module. (5) Small ports for inlet tubing (turbidostatic, pH-stat tubing, etc.).

(B) – (1) Cooling coil + tubing. (2) Heater port. (3) Port with silicon plug. (4) Plastic nuts holding gas sparging system. (5) Small ports for outlet tubing leading into outlet pump (turbidostatic, pH-stat tubing). (6) Check valve = pressure relief valve. (7) Temperature sensor.

3.2 ILLUMINATION MODULE

The LED panel is mounted adjacent to the cultivation vessel and includes the necessary electronics as well as active and passive ventilation. The high-power light emitting diodes generate a highly uniform irradiance flux that can be controlled in the range 0 – 800 (or up to 2,200) $\mu mol.m^{-2}.s^{-1}$ PAR. The irradiance can be dynamically modulated by the PBR software. Spectra are shown in Fig. 1.

The standard version of the PBR (25 or 100 l) incorporates a bi-color LED panel (cool white - red) that provides a maximum irradiance intensity of up to 800 $\mu mol.m^{-2}.s^{-1}$ (white 500 / red 300 $\mu mol.m^{-2}.s^{-1}$). Each light channel is controlled independently in intensity and phases.
The enhanced LED panel is available only for 25 l PBR and provides illumination of up to 2,200 $\mu mol.m^{-2}.s^{-1}$ (white 1,500 / red 700 $\mu mol.m^{-2}.s^{-1}$).
For more information about available LED panels, please refer to chapter: Versions of Large-Scale PBR.

3.3 CONTROL UNIT

Control unit is a rack comprising control electronics that include main Power controller and controllers for individual modules (depending on the configuration: Power, Photobioreactor, Light, Fluorometer, and Probe controller). Moreover, control computer with pre-installed PBR Control Software and Ethernet switch are integral part of the storing rack. The Control unit may accommodate also air pump with bubble interruption valve, pumps belonging to the Fluidic modules, Gas Mixing System GMS 150, medium reservoir, etc.
The inner equipment of the control rack is accessible through the front door. Each controller has its own separate switch and control LEDs inbuilt in the front side. The ports for communication and power cables of sensors/accessories are built into the controller's rear side that is accessible when the entire rear wall of the Control unit is unscrewed.
The storing rack is equipped with wheels for easy manipulation. There is a socket for the cable routing placed at the bottom part of the rack and ports for tubing (aeration gas, liquids to maintain turbidostatic of pH-stat cultivation) at the side-walls. A touchscreen of the control computer is inbuilt in the rack's front door. For Control unit description, refer to Fig. 6

	For further description following simplification is used: • data cable = communication cable between sensor/regulator and its controller • communication cable = communication cable between controller and control computer
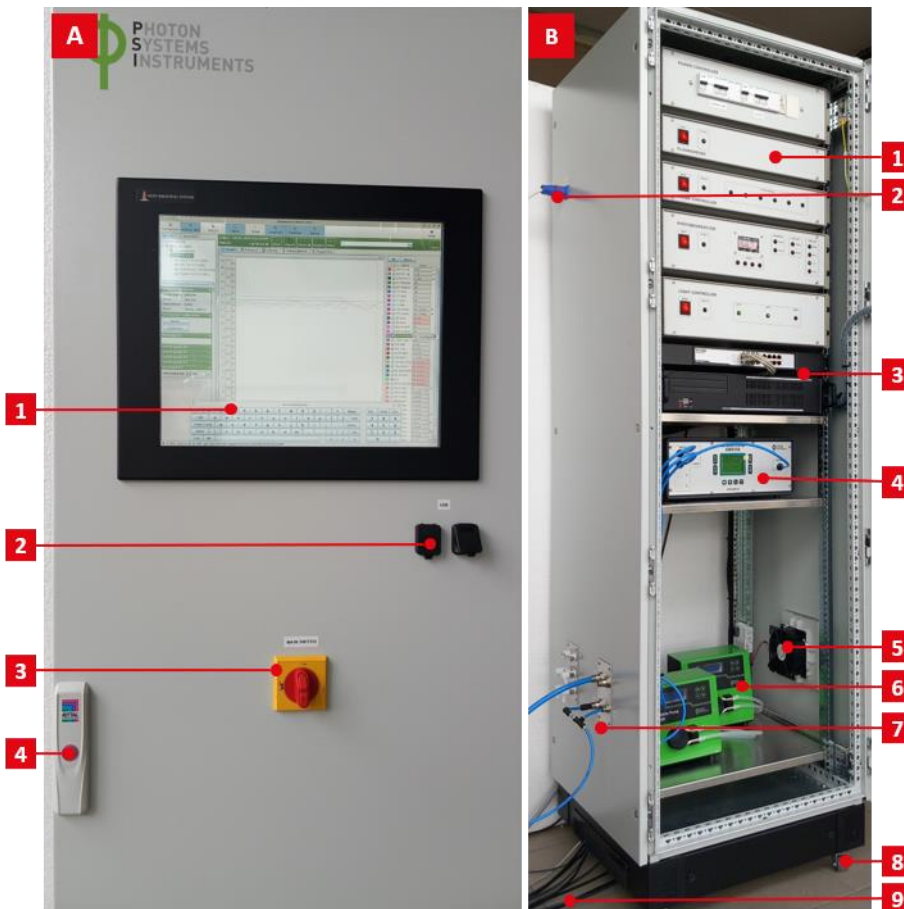
Fig. 6 Control unit.

(A) - (1) Touchscreen. (2) USB ports for mouse, keyboard, USB flash drive. (3) Main switch. (4) Lock. (B) - (1) Controllers. (2) Heater cable. (3) Ethernet switch + control computer. (4) GMS 150. (5) Ventilators. (6) PP600 for pH-stat module. (7) Aeration tubing. (8) Wheels. (9) Cables.

3.3.1 POWER CONTROLLER

The fuses of the cultivation system are located in the main Power controller (Fig. 7).
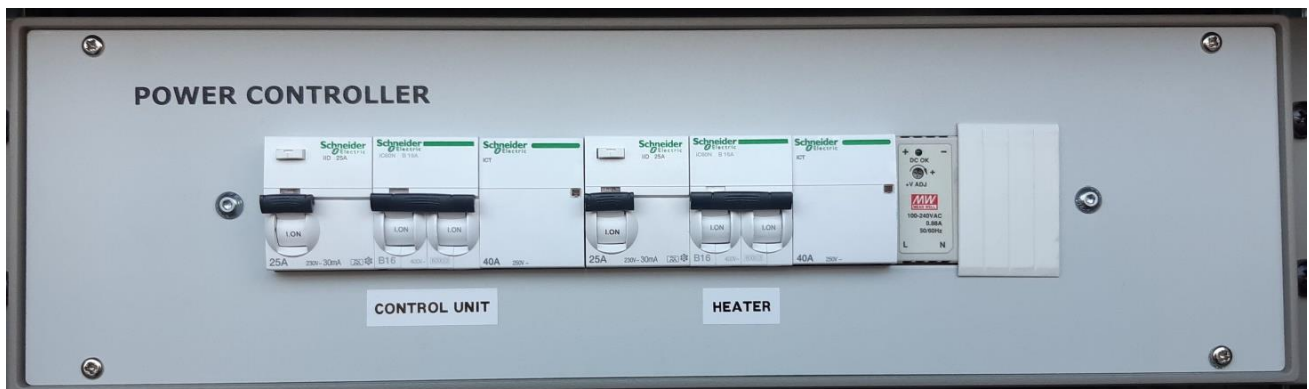

Fig. 7 Power controller.

3.3.2 PHOTOBIOREACTOR CONTROLLER

Photobioreactor controller regulates the temperature in the cultivation vessel and controls pump system and water level sensor (Fig. 8).

Fig. 8 Photobioreactor controller.

(1) Power switch (2) Status indication – flashes when communication is in progress. (3) Active pumps. (4) Thermoregulator – displays current temperature with heating and cooling indication. (5) Level sensors – displays status of solution level in stock canisters and of water level in photobioreactor.

3.3.3 LIGHT CONTROLLER

Light controller handles regulation of light intensity in the bioreactor. It has two individual channels each capable to provide up to 500 W of electrical power.

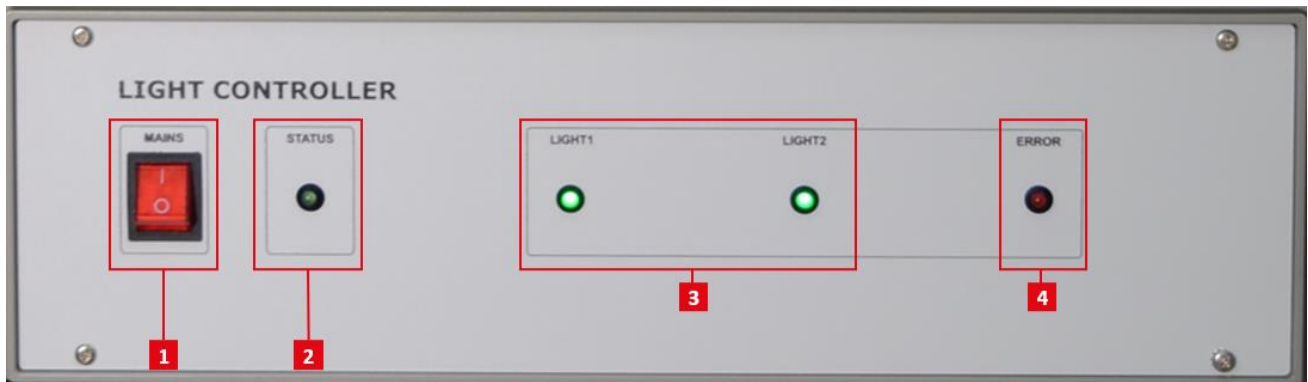**Fig. 9 Light controller.**

(1) Power switch. (2) Status indication – flashes when communication is in progress. (3) Active light channels. (4) Error indication – overheating indication.

3.3.4 FLUOROMETER CONTROLLER
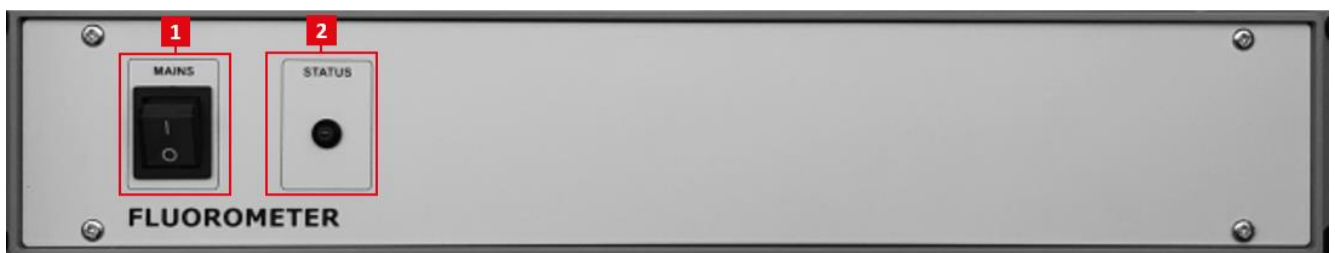
Fluorometer controller operates the Optical module.

Fig. 10 Fluorometer controller.

(1) Power switch. (2) Status indication - flashes when communication is in progress.

3.3.5 PROBE CONTROLLER

Probe controller (Fig. 11) handles communication between Control unit and Mettler Toledo ISM sensors. It is able to communicate with all ISM sensors that are currently on the market. Up to 6 different sensors can be connected.

Fig. 11 Probe Controller.

(1) Power switch. (2) Status indication – flashes when communication is in progress. (3) Active sensor indication – shows connected sensors.

3.3.6 CONTROL COMPUTER + ETHERNET SWITCH

The entire cultivation system is controlled by an industrial computer with Linux operating system and pre-installed PBR Control Software (Fig. 12-A). The computer is connected to the touchscreen interface (Fig. 12-B). The cultivation system can be set locally or using remote access. More information about the installed PBR Control Software GUI and remote access is described in the PBR SW manual.

Fig. 12 (A) Ethernet switch and Control computer. (B) Touchscreen interface.

3.4 AERATION MODULE

The Aeration module consists of plastic U-tube sparger with aeration T-tubing, aeration pump providing sparging gas, and bubble interruption valve. The polyethylene sparger with 50 μm diameter pores is placed on the vessel bottom to provide fine aeration and mixing of the entire culture volume. The aeration pump SECOH MKC-510V can optionally be replaced by the GMS 150 providing accurate flow rate and gas composition. The bubble interruption valve stops the aeration during the OD measurements to avoid the interference of OD measurements by bubbles. Aeration pump and bubble interruption valve are located in the Control unit.

The external compressed gas source (air, CO_2, etc.) are not part of the delivery.

The aeration system as a whole is linked as follows: air source (aeration pump or GMS) >> bubble interruption valve >> gas port in the Control unit >> aeration T-tubing >> U-tube aeration sparger.

For more information about the Aeration module installation, please refer to chapters: PBR Cultivation Vessel Installation, Air Pump Installation and Gas Mixing System GMS 150.

4 INSTALLATION

4.1 PRE-INSTALLATION REQUIREMENTS

Please ensure following conditions for PBR installation:
- sufficient space around PBR (area occupied by the devices: 1 × 1 m for 25 l PBR; 2 × 1 m for 100 l PBR, 1 × 1 m for PBR control rack, 1 × 1 m for Cooling unit)
- easy and safe access to electricity (PBR + Control unit 2 × 220 V/16 A, Cooling unit 1 × 220 V/16 A)
- access to water, preferably to distilled water
- drainage for waste water
- compressed air and CO_2 for GMS 150. GMS input pressure ca. 3 - 5 bar. GMS connectors fit to 6 × 1 mm Parker Polyflex Presto TPU tubing

Additional recommended equipment:
- bench or table for placing individual parts, sensors, etc.
- tile or smooth surface around the PBR to facilitate cleaning around the unit
- barrels for medium, buffer, biomass
- soft cleaning brushes with long holder, squeegee mop
- tubing cutter

4.2 FOAM TRAP INSTALLATION

Screw the holder of foam trap onto the prepared mounting brackets (Fig. 13-A, B). Attach the lid to the handle and install the plastic bottle (Fig. 13-B, C). Use Parker Polyflex tubing to connect PBR gas output with the Foam trap (Fig. 13-D).
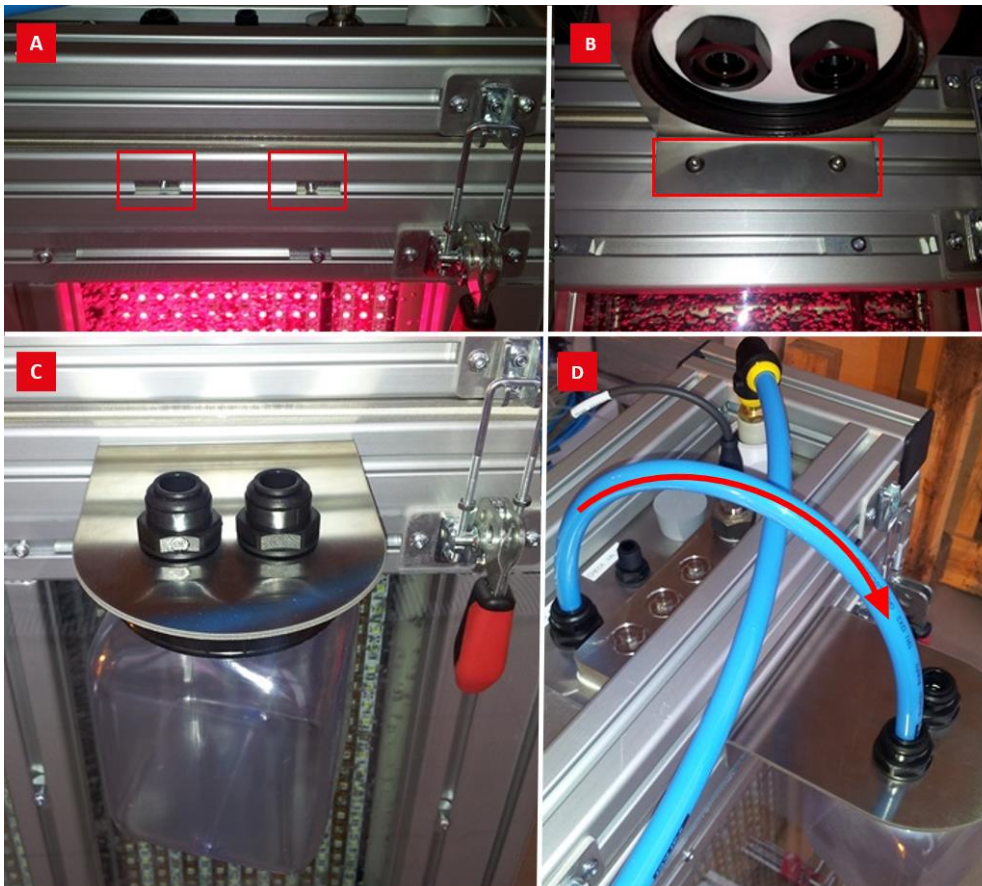

Fig. 13 Foam trap installation.

(A) Mounting brackets (B) Holder with lid attached to the PBR construction (C) Plastic bottle (D) Connection of PBR gas output with Foam trap.

4.3 PBR CULTIVATION VESSEL INSTALLATION

Following items need to be connected and/or installed: LED panel, Foam trap (refer to the previous chapter), U-tube aeration system, Optical module (optional), Water level sensor, Cooling coil (optional), Heater, Temperature sensor.

ℹ️	Two persons are recommended to manipulate with lid and U-tube aeration system of 100 l cultivation vessel. Each of them holds one side of the lid (or aeration system) and lifts it simultaneously.

⚠️	Be careful when manipulating the sensors and the aeration system as they are fragile and can be easily damaged. The water level sensor in particular is extremely fragile. Do not touch the forks and store them upside down in a safe place when not in use.

LED panel installation: unscrew back panel from the Control rack. Pull the LED panel cable through the rack socket just like the other cables. Connect the cable with Light controller according following picture (Fig. 14). Secure the cable with cable glands to the rack construction. Install the back panel of the rack.

Fig. 14 LED panel installation.

Aeration system installation: insert the U-tube sparger in the empty cultivation vessel. Place silicone gasket and lid on cuvette with sparger tubes passing through plastic nuts. Lock all secure clips and tighten the plastic nuts. Assembly external aeration T-tubing as shown below (Fig. 15-A) and connect it to the sparger tubes (Fig. 15-B) and to the gas outlet of the Control unit Fig. 15-C.
An air filter can be inserted in the aeration tubing before splitting the tubing in two branches (prior the T-connector). Use a large air filter and check it regularly to prevent its clogging.
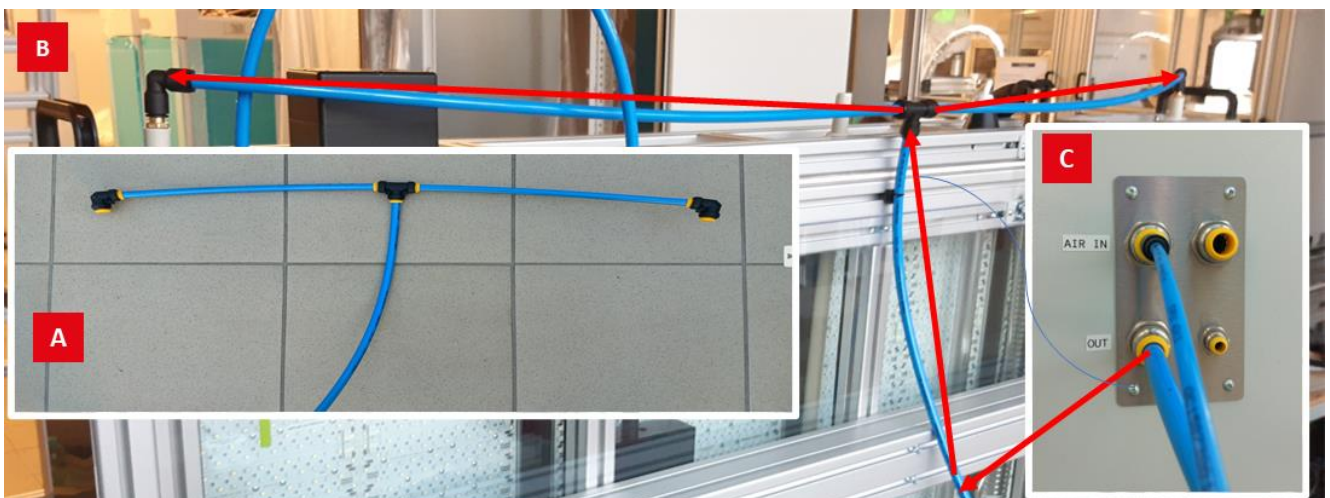

Fig. 15 Aeration system installation.

(A) Assembling of aeration T-tubing. (B) Connection of aeration tubing with Cultivation vessel. (C) Connection of the aeration tubing with the gas outlet of the Control unit: tubing from OUT port leads into the Cultivation vessel.

Water level sensor installation: place silicone gasket and water level sensor in the correct lid compartment. Fix it with 8 screws. Connect the sensor to the Control unit with the appropriate cable (Fig. 16).
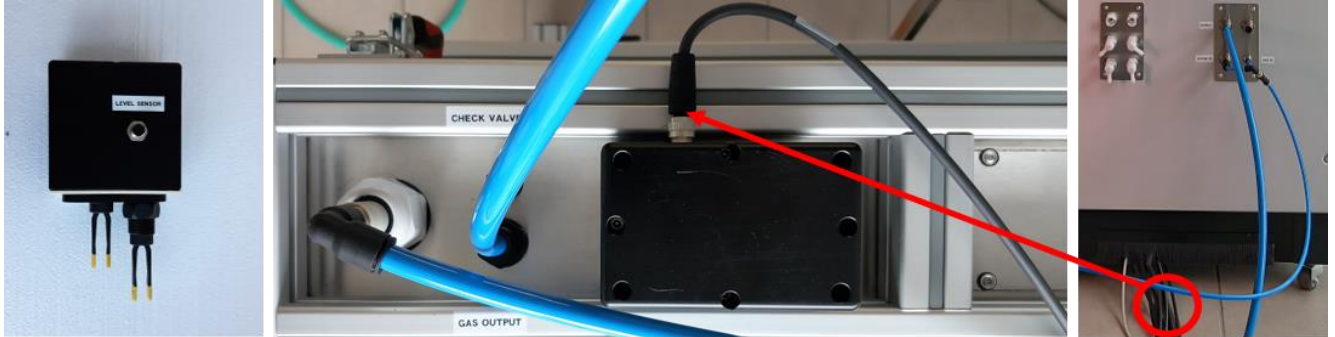

Fig. 16 Water level sensor installation.

	The water level sensor must be installed and working correctly because it provides a safety function: the sensor shuts off the thermoregulation when the water level in the cultivation cuvette is low, since heat emitted from the unsubmerged heating element may damage the glass cultivation cuvette.

	Be careful when handling the water level sensor as it is extremely fragile. Do not touch the forks and store them upside down in a safe place when not in use.

Cooling coil (optional) installation: insert cooling coil with silicone sealing and plate in the appropriate lid port. Fix the plate with 4 screws. Connect the quick connect fittings and hoses. Connect cooling hoses to the Cooling unit. Use insulating layer wrapped around the hoses. The hose order does not matter (Fig. 17).
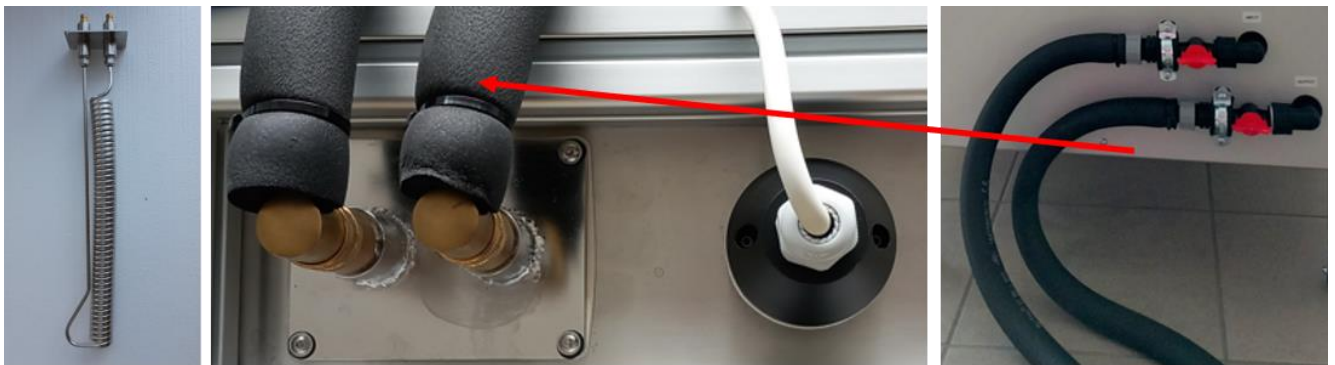

Fig. 17 Cooling coil installation.

Heater installation: insert heater with silicone sealing in the appropriate lid port. Fix the heater with 2 screws. Connect heating cartridge to the Control unit with the appropriate cable (Fig. 18).

Fig. 18 Heater installation.

⚠️	When the heating element heats, it must be submerged along its entire length, otherwise the emitted heat may damage the glass cultivation cuvette. Low water level is indicated by the water level sensor automatically.

Temperature sensor installation: Screw temperature sensor in the right port of the sensor compartment. Tighten it well, use Teflon sealing tape if necessary. Connect sensor to the Control unit with the appropriate cable (Fig. 19).

Fig. 19 Temperature sensor installation.

4.4 AIR PUMP INSTALLATION

Place the air pump in the Control unit and connect the pump to the prepared power cord. Assembly the aeration tubing as shown in the images: the aeration tubing leads from a gas source (air pump or GMS 150) through the bubble interruption valve and the Gas outlet (in the side wall of the Control unit) to the U-tube sparger (placed in the cultivation vessel).

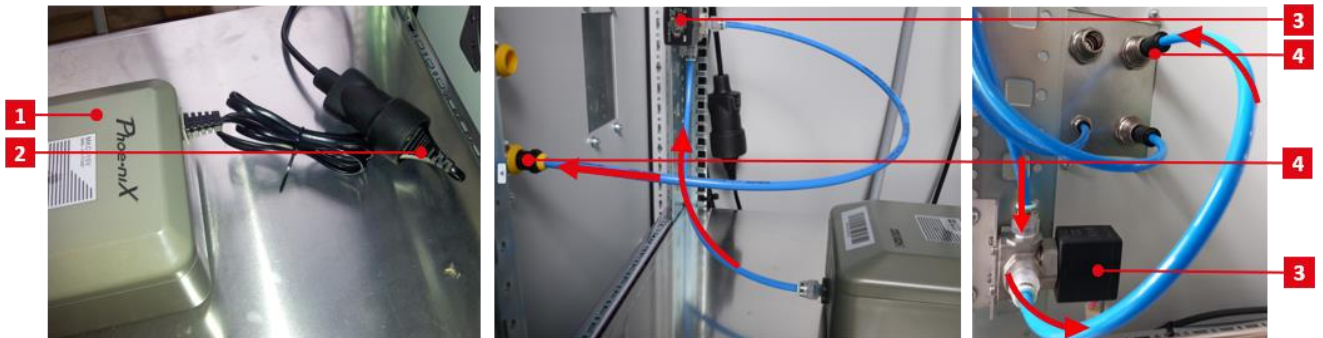| ⚠️	The installation of the tubes leading into and out of the bubble interruption valve depends on the PBR configuration and the orientation of the bubble interruption valve. To install the tubes in the right direction, follow the indication on the bubble interruption valve.

Fig. 20 Air pump installation

(1) Air pump. (2) Power cord. (3) Bubble interruption valve. (4) Gas outlet.

4.5 ETHERNET INSTALLATION

Connect the Ethernet cable coming out of the rack socket to your local network. Please note that Wi-Fi is not available.

5 OPTIONAL ACCESSORIES

5.1 OPTICAL MODULE

Optical module includes pulse amplitude modulated (PAM) fluorometer and densitometer. The PAM fluorometer measures fluorescence parameters (continuous fluorescence yield, Photosystem II quantum yield) with blue and red excitation spectrum. Blue excitation light (455 nm) is intended for chlorophyll excitation, i.e., for measuring chlorophyll fluorescence in algal cultures. Red-orange excitation light (620 nm) is intended for excitation through phycobilin and is suitable for measuring in cyanobacteria.

The densitometer determines optical density (OD) in two wavelengths 680 nm and 720 nm. OD 680 nm represents light scattering and chlorophyll absorption. OD 720 nm represents light scattering.

The densitometer has to be calibrated before each experiment to blank using blank medium and PBR Control Software.

Optical module installation

Unlock all clips and remove the lid from the cultivation vessel. Insert the Optical module into the lid, place the gasket and cover plate on the lid and secure the plate with all screws. Screw on the sleeve. Connect the sensor to the Control unit with the appropriate cable (Fig. 16).

Fig. 21 Optical module installation.
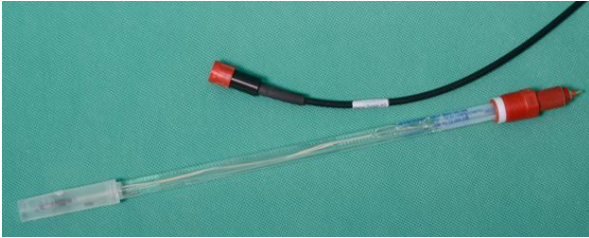
5.2 ELECTRODE MODULE

pH Module

Fig. 22 pH sensor with cable

The module consists of Probe controller placed in the Control unit, sensor and cable (Fig. 22). InPro3253i/SG/325 single rod measuring cell from METTLER TOLEDO (www.mt.com) is supplied as low maintenance, autoclavable and pressure resistant pH electrodes with a factory filled pre-pressurized liquid electrolyte and a reference electrolyte together with a silver ions barrier. Measurement disturbances caused by earth potentials are avoided through employment of a "solution ground" option available with InPro3250SG version. For more information, please refer to manual provided by electrode manufacturer.

dO$_2$ Module

Fig. 23 dO$_2$ sensor with cable

The module consists of Probe controller placed in the Control unit, sensor and cable (Fig. 23). dO$_2$ optical sensor InPro6860i/12/320 from METTLER TOLEDO (www.mt.com) is used for oxygen measurement. Operation of the InPro6860i/12/325 sensor is based on optical fluorescence quenching. For more information, please refer to manual provided by electrode manufacturer.

dCO$_2$ Module

Fig. 24 dCO$_2$ sensor with cable

The module consists of Probe controller placed in the Control unit, sensor and cable (Fig. 24). InPro5000i/12/320 sensor from METTLER TOLEDO (www.mt.com) with integrated temperature probe is used for potentiometric CO$_2$ concentration measurement. The InPro5000i sensor employs a gas permeable silicone membrane which is tightly stretched around a special engineered flat pH membrane. CO$_2$ partial pressure can be calculated using the pH and the temperature. For more information, please refer to manual provided by electrode manufacturer.

Electrodes installation

Screw the electrodes into the lid and connect them with the Control unit using the appropriate communication cables (Fig. 25). pH and dCO$_2$ cables are interchangeable.

Fig. 25 Electrodes installation.

Sensor calibration

All electrodes are based on Digital ISM technology (Intelligent Sensor Management®) from Mettler Toledo. This technology allows to calibrate the sensors in laboratory using provided USB dongles and free software iSenseLight. For sensors calibration, follow the instructions in iSenseLight software. For more information, please use following link:

http://uk.mt.com/gb/en/home/products/ProcessAnalytics.html
https://www.mt.com/de/en/home/library/software-downloads/process-analytics/iSense_light.html

Sensor maintenance

For electrode maintenance, please refer to manual provided by electrode manufacturer.

- The pH electrode is autoclavable but the manufacturer states that autoclaving deteriorates it rapidly. If possible, use warm water with a detergent or chemical disinfectants (for example 75% ethanol, 1 M HCl) for its cleaning.
- O_2 and CO_2 electrodes are made of high-quality stainless steel, grade 316, which is autoclavable. Yet, we recommend you to keep autoclaving to a reasonable minimum.
- Be aware that, although the O_2 and CO_2 electrode are made of high-quality stainless steel, a very aggressive growth medium can cause its corrosion. Consult your specific conditions with the electrode manufacturer.

⚠️	Refer to the manufacturer's manual for calibration, maintenance and storage of sensors when not in use. Improper handling may damage the electrodes.

5.3 GAS MIXING SYSTEM GMS 150

GMS 150 can be used instead of aeration pump to provide gas sparging of the biomass, refer to Fig. 26 for GMS description. The Gas mixing system creates precise mixture of air and CO_2. The external gas sources are not part of the delivery. GMS should be placed the Control unit.

Fig. 26 Gas Mixing System GMS 150: description.

(1) CO₂ input. (2) Air/N2 input. (3) Mixture output. (4) User interface. (B) – (1) Power cable, main switch. (2) Communication cable.

⚠️	Do not change any values directly using front instrument keyboard. Use the PBR Control Software GUI to set gas concentration and flow rate.

BEFORE OPERATING THE GMS 150 READ THE FOLLOWING INSTRUCTIONS AND WARNING CAREFULLY:

- To operate a gas handling equipment, you should be trained in its proper use or be under competent supervision.
- The device is primarily intended to mix CO_2, N_2 and air. Mixing of other gasses must be always consulted with manufacturer.
- Pressure regulator must be always used when connecting the GMS 150 with the gas tank. Never connect the gas tank directly to the GMS 150.
- Do not apply pressure in the gas inputs until the GMS 150 is switched on and fully operational.
- Gas input pressure should be 3 – 5 bars. Gas input pressure cannot exceed 8 bars.
- Input gas must be free of oil, water or any other foreign substances.
- If working in wet conditions, dehumidifier must be used to prevent condensation of water in tubes and the system.
- Avoid flooding of the GMS 150 valves by proper device installation and operation. Place the GMS over the cultivation system. If the GMS is not in operation, disconnect the tubing to prevent liquid backflow.
- Follow the basic safety rules for work with CO_2 and other hazardous gases.
- Never heat or expose a gas tank or gas handling equipment to temperatures above 50 °C (120 °F).

⚠️	Please read the separate Instruction Guide for GMS 150 before operating the device. Pay attention especially to chapter Safety precautions. If you are not sure about something in the manual, contact the manufacturer for clarification.

GMS 150 installation

1. Open front door of the Control rack and place the GMS 150 on the shelf. Connect power and communication cable in the device connectors on the rear side of device (Fig. 27-A). If the cables are not accessible, unscrew back panel of the Control rack to reach them. Switch on the device through the main switch on the rear side.
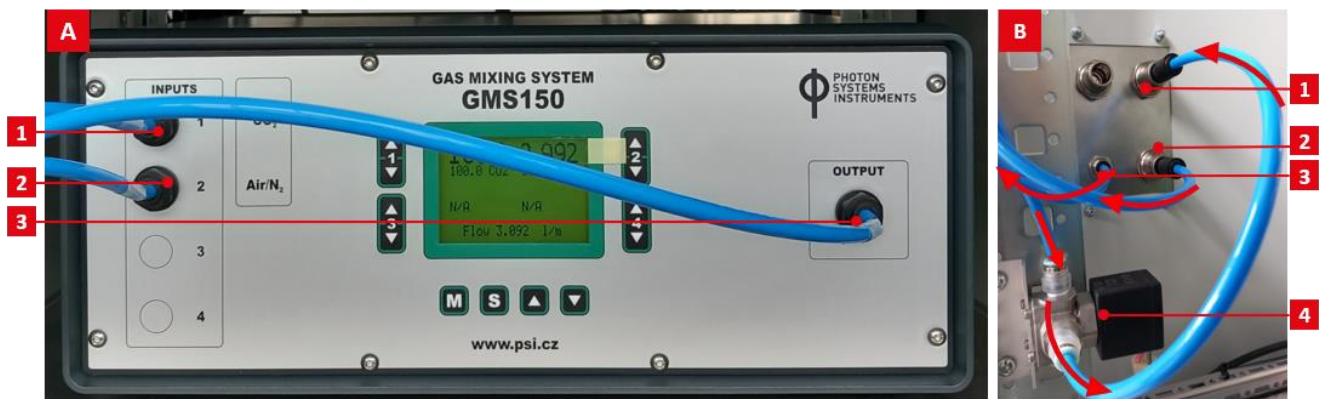2. Connect gas tubing as shown on the following figure (Fig. 27-B).

Fig. 27 GMS 150 installation.

(A) Gas Mixing System (1) CO_2 input. (2) Air/N2 input. (3) Mixture output. (B) Control unit: gas ports and bubble interruption valve (1) Gas OUT port. (2) Air IN port (tube leads into GMS Air/N2 input). (3) CO_2 IN port (tube leads into GMS CO_2 input). (4) Bubble interruption valve.

3. Connect tubing from external pressurized Air source through Air IN port (Control unit) into GMS Air input. Don't switch on the Air unless GMS is switched on and fully operational. The input pressure should be ca. 3 - 5 bars.
4. Connect tubing from external pressurized CO_2 source through CO_2 IN port (Control unit) into GMS CO_2 input. Don't switch on the CO_2 unless GMS is switched on and fully operational. The input pressure should be ca. 3 - 5 bars.
5. Connect tubing from GMS mixture Output port into bubble interruption valve.
6. Connect tubing from bubble interruption valve in the Gas OUT port (Control unit).

5.4 COOLING MODULE

Cooling module description

The Cooling module extends the temperature range of the cultivation system down to 15 °C at the ambient temperature around 20 °C. Proper function of the Cooling module requires installation of cooling coil, heater, temperature sensor and water level sensor in the cultivation cuvette (refer to chapter 3.)

The Cooling module consists of Hailea water chiller that cools down also water in the additional water reservoir placed in the upper compartment of the Cooling unit. System of two pumps ensures internal water circulation between Hailea chiller and water reservoir and external circulation between Cooling unit and cultivation cuvette. The Control unit uses feedback from the PBR temperature sensor to automatically operate the entire cooling system.
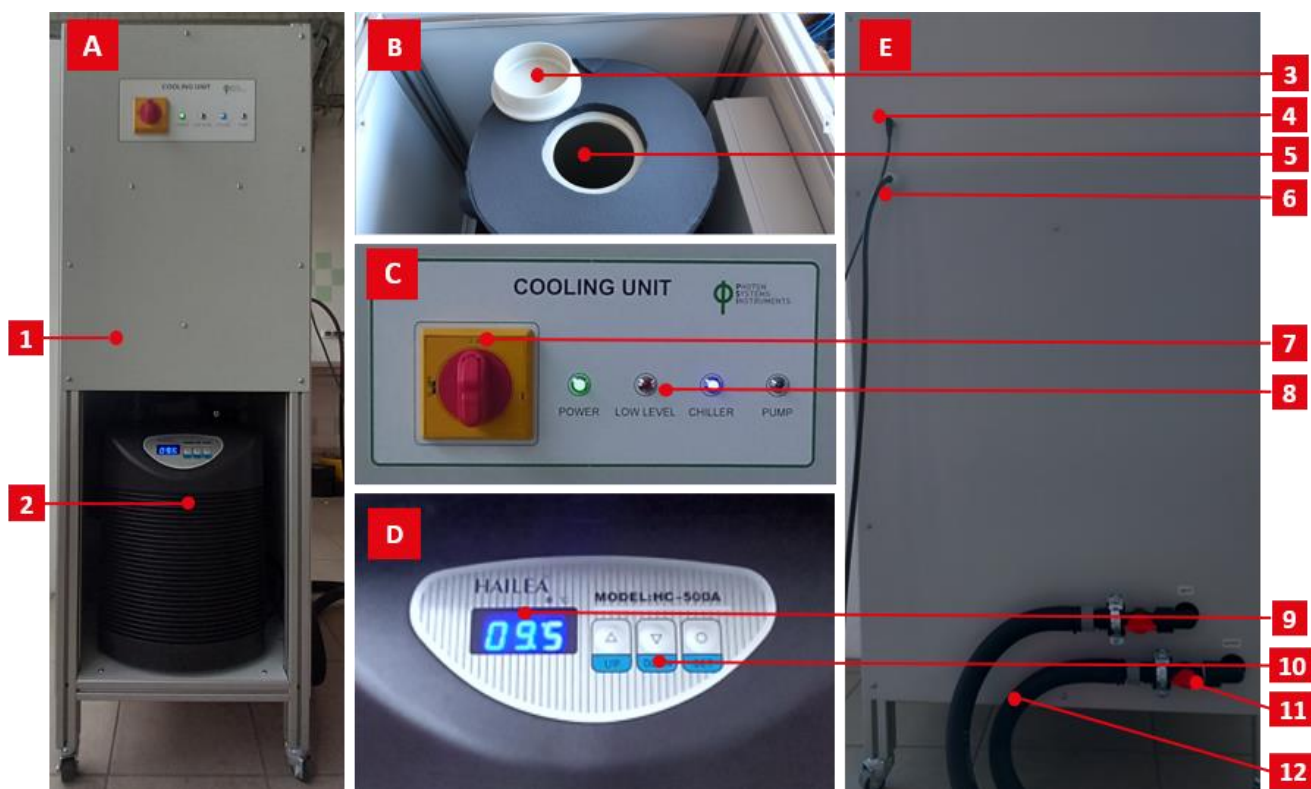

Fig. 28 Cooling unit: description.

(A) Cooling unit. (1) Upper compartment with water reservoir. (2) Hailea chiller.
(B) Upper compartment. (3) Lid. (5) Insulated water reservoir with inner pump.
(C) Main control panel of the Cooling unit. (7) Main switch. (8) Status indicator – when flashes the item is active.
(D) Hailea control keyboard. (9) Current temperature of the chiller's water bath. (10) Control keyboard.
(E) Side wall of the Coolin unit. (4) Communication cable between Cooling and Control unit. (6) Power cable. (11) Manual valve. (12) Insulated hoses leading cool water in the cooling coil placed in PBR cultivation vessel.

Cooling module installation

1. Install cooling coil in the cultivation vessel as described in Fig. 17.
2. Connect main power cable of the Cooling unit to 220 V/16 A.
3. Connect the Cooling unit with the Control unit: the communication cable comes out of the bottom socket of the Control unit.
4. Wrap an insulating layer around the cooling hoses and connect them to the closed water valves placed on the side wall of the Cooling unit. Closed valves - valves in vertical position.
5. Connect cooling hoses to the cooling coil (the order of the hoses does not matter).
6. Fill the upper water reservoir of the Cooling unit with distilled water. The water level should be ca. 10 cm below the top edge.
7. Open the water valves (= valves in horizontal position).
8. Switch on the main switch.

	The Hailea chiller default temperature is set to 5 °C. The lower temperature can cause the water in the chiller to freeze.

5.5 FLUIDIC MODULES

Fluidic system description

The complete Fluidic pumping system (Turbidostat module + Chemostat module + Level stat) consists of two diaphragm pumps (for medium feed to keep the OD at a predefined level and for biomass overflow) and two peristaltic pumps PP600 (for an automatic acid and base addition to keep pH at a predefined level). The output diaphragm pump is common for all mentioned modules. For a scheme of Fluidic system, please refer to Fig. 29.

The diaphragm pumps are placed on the side walls of the cultivation vessel, the peristaltic pumps are placed in the Control rack.

The Fluidic pumping system can run in three regimes which must be set in the PBR Control Software:
- Turbidostat – selected optical density is regulated on predefined value (medium canister). PBR Control Software: OD Regulator + Switch pump with ID 0 + 1.
- Chemostat – pH is regulated on predefined value using acid or base (buffer canisters). PBR Control Software: pH Regulator acid + pH Regulator base + P-Pumps with ID 1 and 2.
- Level stat – medium level in the photobioreactor is kept on a constant value to eliminate evaporation (medium canister).
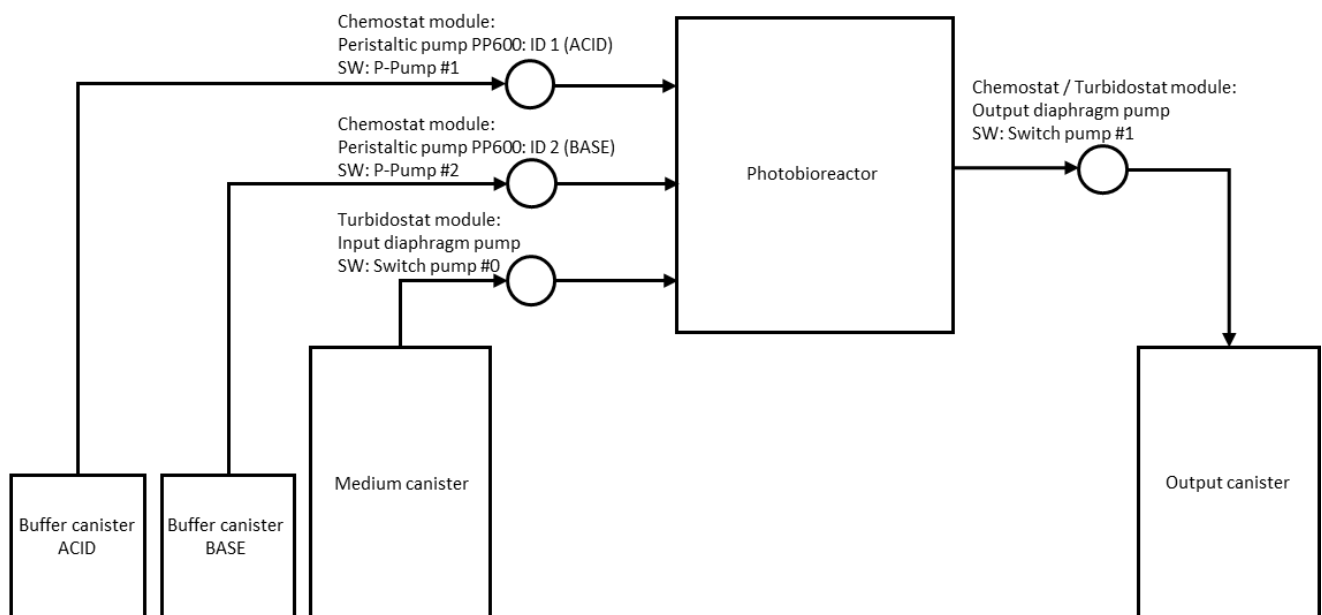

Fig. 29 Scheme of fluidic system.

The Level stat module is an integral part of the fluidic system. It contains the water level sensor and can use the pumps included in the Turbidostat or Chemostat module, if purchased. Primary function of the Level stat is safety: if the water level sensor detects a low water level in the cultivation cuvette, the heater is switched off automatically to prevent a damage to the system. Depending on the available diaphragm pumps and setting in the PBR Control Software, the Level stat can be run in four modes:
- without software setting >> if the water level is too low, the heating is stopped automatically. No additional pump/SW setting is needed.
- High only >> water is automatically pumped out from the cultivation cuvette if the water level becomes too high (based on the feedback from the water level sensor). Only output diaphragm pump is needed for this mode.
- Master >> water level in the cultivation cuvette is automatically kept at a constant level based on the feedback from the water level sensor. The system uses input and output diaphragm pumps to pump in the medium if the water level is too low and pump out the excess biomass if the water level is too high.
- Slave >> this mode has to be used if the turbidostatic cultivation is set. In such a case, the Level stat is subordinate to the feedback from the water level sensor and OD sensor simultaneously.

For more details, see chapter: PBR Control Software: Level regulator.

⚠️	The primary function of the Level stat is safety: if the water level sensor detects a low water level in the cultivation cuvette, the heater is switched off automatically to prevent a damage to the system.

Fluidic system installation

1. Bolt the turbidostatic diaphragm pumps on the side walls of the PBR construction. Input pump on left, discharge (output) pump on right (when looking at PBR from front).
2. Connect pumps with the Control unit using cables labelled "PUMP IN" and "PUMP OUT" (Fig. 30): unscrew back panel from the Control rack. Pull the pumps cables through the rack socket just like the other cables. Connect the cable with Photobioreactor controller according following picture. Secure the cable with cable glands to the rack construction. Install the back panel of the rack.

Fig. 30 Turbidostatic (diaphragm) pump installation.

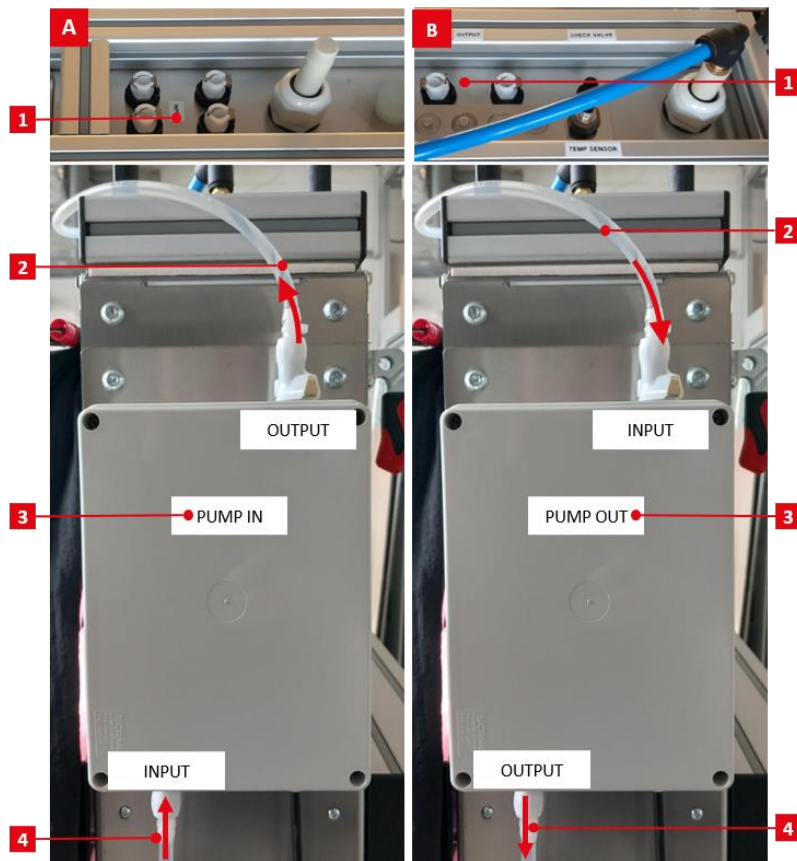3. Connect tubing as illustrated in the
4. Fig. 31.

Fig. 31 Turbidostatic (diaphragm) pumps connection.

(A) Pump IN. (1) Input ports in PBR lid. (2) Tubing leading into inputs ports. (3) Pump IN for medium feeding. (4) Tubing from stock medium cannister.
(B) Pump OUT. (1) Output ports in PBR lid. (2) Tubing leading out of output ports. (3) Pump OUT for surplus medium. (4) Tubing into waste canister.

> For detailed Peristaltic Pump PP600 description, installation and operation, please see the User Guide related to the PP600 that can be found on https://photo-bio-reactors.com/products/photobioreactors/#download.
> If the PP600 is connected via AUX communication cable with the Control unit, do not change any values directly using front instrument keyboard. Use the PBR Control Software GUI to set the direction of rotation and flow rate.

5. Place the peristaltic pumps labeled Acid and Base on the bottom shelf of the Control unit and connect them to the power source using the power supply cord. Each pump has its own power source, the order does not matter.
6. Switch on the pumps and set ID = 1 for Acid pump and ID = 2 for Base pump. For setting, go to the main control display >> Settings >> Pump ID.
7. Set direction of rotor rotation using the main control display for each pump. Go to the main control display >> Settings >> Rotate.
8. Cascade the pumps using the AUX cable.
9. Place the Norprene peristaltic tubing around the rotor and connect the Luer connectors and silicone tubing leading to the ports in the side wall of the Control rack.

> ⚠ Pay attention to the direction of PP600 rotation (direction of the line), because the following connection of the silicone tubes leading from canisters with acid and base stock solution and into the cultivation vessel depends on it. From this reason, the ports in the side wall of the Control rack are not labelled and the user has to check this himself.

10. Connect the tubing leading from acid and base pumps in the inlet ports placed in the cultivation cuvette lid. Pay attention to the direction of the line.
11. Connect the tubing from buffer canisters (acid and base) into the side wall of the Control rack. Pay attention to the direction of the line.
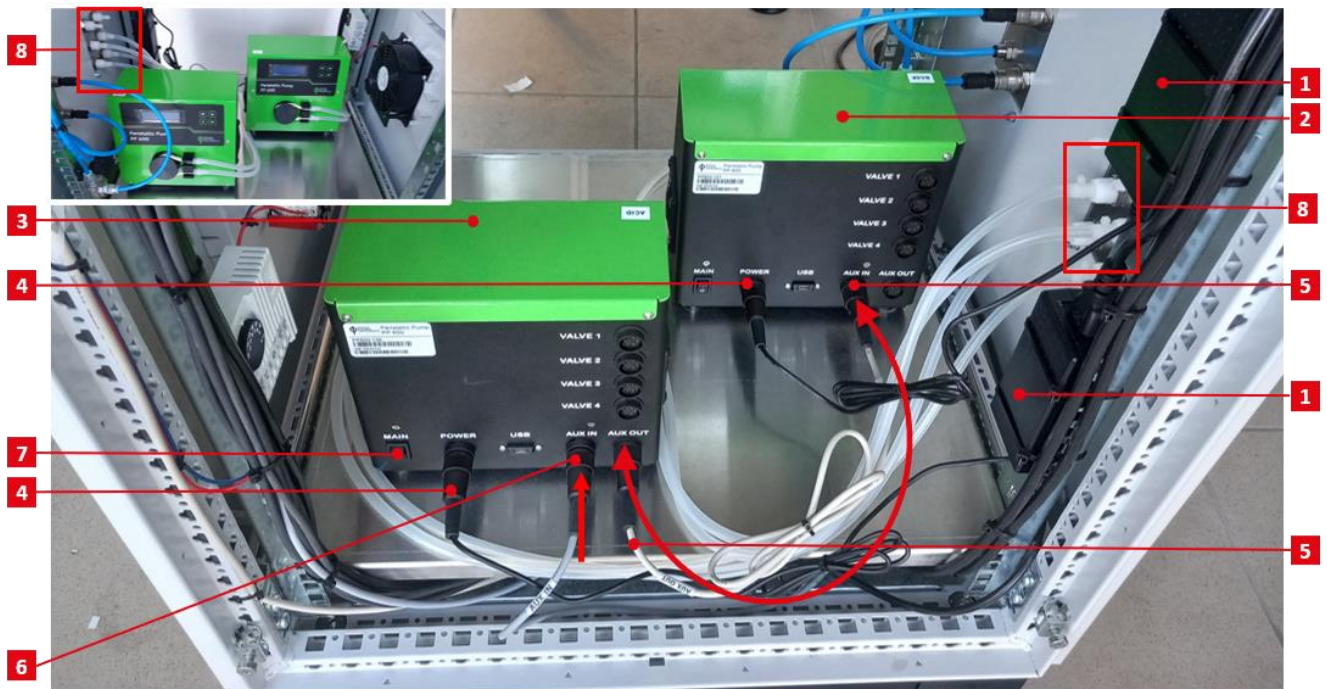

Fig. 32 Peristaltic pumps installation.

(1) Power source (2) PP600-Base. (3) PP600-Acid. (4) Power cord. (5) AUX IN + AUX OUT = pump cascade, (6) AUX in = AUX communication cable connecting the pump system with Control unit. (7) Main switch. (8) Fluidic ports.

6   PBR Cultivation Vessel Cleaning

In most cases, hot water (60 – 70 °C), standard detergent (0.25 % sodium hypochlorite) and thorough mechanical cleaning are sufficient to keep the equipment clean and uncontaminated.
Diluted citric acid or mild bleach can be used to remove deposits in the cultivation vessel (3 - 5% citric acid with warm water to remove inorganic deposits and 5 – 10% bleach to remove organic deposits). Always follow safety instructions when working with chemicals.

The gas sparger can be cleaned mechanically and with the above-mentioned chemicals as well. It is also autoclavable. Chemical treatment and autoclaving of the sparger will shorten its lifetime.
The silicon sealing is autoclavable.
To clean the optical module, thorough mechanical cleaning and 75% ethanol are sufficient to keep the optical module clean and uncontaminated. It is recommended to use a soft brush or cloth to clean the inner glass chamber to avoid scratching.
To clean the stainless-steel components (lid, heater, cooling coil, etc.) use hot water (60 – 70 °C), standard detergent and gentle mechanical cleaning (avoid scratching of the components). Also, 75% ethanol can be used.
Separate lid without plastic connectors is autoclavable, but this should only be done occasionally

The cultivation vessel is not autoclavable.
The cleaning process can be adjusted to the organisms being cultivated – if the target organisms prefer an acid environment, then cleaning with a basic agent is recommended. The cultivation vessel should not be exposed to strong acid/base or their vapors.
After cleaning, it is very important to rinse off any detergent or medium residues from cuvette or individual items.

7   Quick Start Guide

7.1   Turning on the Cultivation System
1.   Plug the main power cable of the Control unit into the socket.
2.   Turn on the Control unit using main switch placed at the doors.
3.   Check the touchscreen – if the control computer is not running, switch it on.
4.   The Bioreactor Client (GUI of the PBR Control Software) will launch automatically (if not, find the Bioreactor Client in the main menu of the operating system and start it).
5.   Make sure, all controllers placed in the Control unit are switched on (including control computer, GMS 150, peristaltic pumps).
6.   After the GMS 150 initialization, plug in the inlet Air and CO_2 tubing and open the reduction valves of the external gas source (3 – 5 bars).
7.   Check the ID of the peristaltic pumps using the PP600 front control display.
8.   Plug the main power cable of the Cooling unit into the socked.
9.   Turn on the Cooling unit using main switch placed at the front side of the unit.
10.   If needed refill distilled water in the water reservoir.
11.   Set the Hailea chiller temperature to 5 °C using Hailea front control keys.
12.   Open the cooling water valves.

7.2   Preparation of the Cultivation
1.   Prepare a sufficient amount of algae inoculum to start the cultivation in 25/100 l PBR in advance. The recommended initial PBR OD680 is around 0.1 for green algae, but it might be possible to use also a biomass with lower density around 0.05. Optimize the appropriate initial density depending on the experimental species and the purpose of the cultivation.
2.   Prepare the PBR for cultivation: clean the cultivation vessel and close its emptying valve. Check all components necessary for PBR operation (heater, water level sensor, cooling coil, sealing, lid, pumps, etc.).
3.   Depending on the PBR configuration and experiment, prepare and install canisters for stock solutions (medium, acid, base) and waste canister.
4.   Insert a gas sparger into the cultivation vessel.
5.   Cover the cultivation vessel with sealing and placed on it lid with installed OD module. Fix the lid with clamps and install other items (heater, water level sensor, cooling coil, temperature sensor, etc.).
6.   Connect all necessary tubing (aeration tubing, fluidic tubing, cooling tubing, etc.).
7.   Prepare sufficient volume of growth medium (volume depends on the vessel capacity) and pour it in to cultivation cuvette. It is recommended to prepare medium or stock solutions in external cannisters, then pour or pump the medium or stock solutions into the cultivation vessel and top up with distilled water as needed. The nutrients in the stock solutions or medium should be dissolved prior use. Leave enough headspace for algal inoculum.

8. Prepare stock solutions (medium, acid, base)
9. Calibrate the sensors for pH, O_2 and CO_2 (external calibration using iSenseLight software)
10. Insert sensors for pH, O_2 and CO_2 in the lid ports.
11. Connect all data/communication cables.
12. Use PBR Control Software to set the experimental conditions (illumination, temperature, etc.), including aeration if the GMS 150 is used.
13. Check the rotation of the PP600 pump using the PBR Control Software and the tubing connection to make sure the flow direction of acid and base is correct.
14. Calibrate the OD module (if needed, the OD module can be calibrated also out of the cultivation vessel in a cannister filled with blank medium).
15. After achievement of the appropriate experimental conditions, add the algae inoculum through the open port using funnel.
16. Start the experiment using PBR Control Software.

7.3 PBR CONTROL SOFTWARE

For detailed information related to the PBR Control Software (PBR SW) please see software manual that can be found on PSI website: https://photo-bio-reactors.com/products/software/#download
In this chapter, just a very limited description of the PBR SW will be provided.

The computer for the photobioreactor control is shipped with a pre-installed Linux Debian operating system as well as with the PBR Control Software. Thus, no installation by the user is, typically, required.
The PBR Control Software consists of two parts:

- The core software (server) controls the photobioreactor system and runs in the background without direct communication with the user. It runs automatically anytime the computer is switched on.
- The graphical user interface (GUI) - Bioreactor Client - connects locally or remotely to the core and facilitates the communication with the user.

After the Linux Debian operating system boots up, the Bioreactor Client will start automatically. If not, the Bioreactor Client can be found in the main menu of Debian OS. To log into the software, use:
Login: bio-reactor
Password: reactor
Please note, that some software function may be accessible only through the administrator account. Please refer to the software manual for more details on available account management.

For description of the main software window, see Fig. 33 including related details below the image.
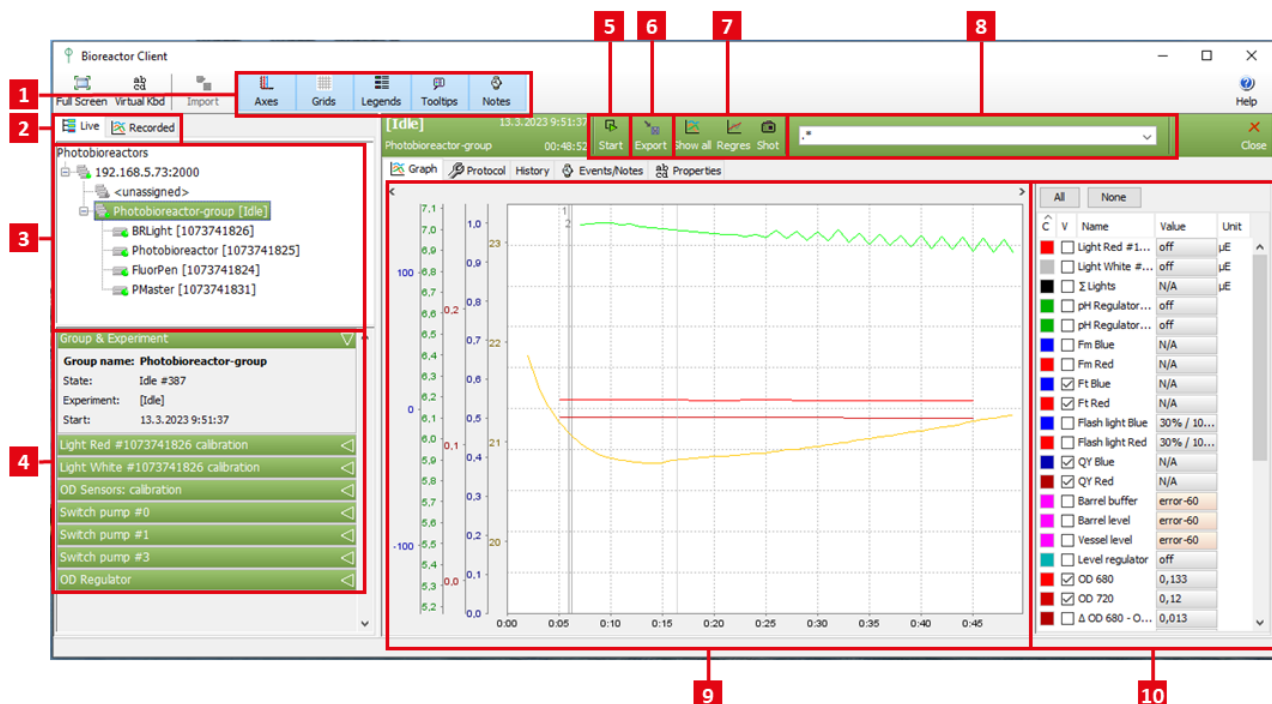

Fig. 33 PBR Control Software: Main window

(1) Visualization tools related to the graph.

(2) Live and Recorded experiments tab: Live tab displays running experiments. Recorded tab displays all finished experiments that are saved in the server database.

(3) Live tree with all connected PBR groups.

There may be multiple Photobioreactors connected with the PBR SW. Each Photobioreactor creates its own group that includes all connected controllers with related accessories/sensors. Green dot means, the controller with accessories is connected and recognized by the PBR SW.

BRLight	>> Light Controller
Photobioreactor	>> Photobioreactor Controller
FluorPen	>> Fluorometer Controller
PMaster	>> Probe Controller

The PBR experiment can be in Idle or Recording mode, the experiment state is displayed in the bricked next to the PBR group (3). In the Idle mode, the visualized data are not recorded or saved. To begin recording data, the Photobioreactor must be in the Recording mode, i.e., the experiment must be started pressing the Start button (5). Only the user who has started the recording or an administrator can make changes to Recording experiment or can stop it.

Prior starting the experiment, it is recommended to calibrate the OD sensor (4) via the PBR SW and set up set up the experimental protocol.

(4) The panel includes details about selected PBR group and experiment, OD calibration, setting of connected pumps flow rates and setting of OD regulator (680 or 720 nm). Depending on the software version, also Light calibration may be included. In the latest software version, the light calibration is included in the local context menu of the related light parameter in the Protocol legend panel (10).

(5) Start/Stop button to start or finish the recording mode.

(6) Data Export to different formats allowing further data processing.

(7) Experiment graph toolbar contains button allowing to zoom in, perform regression and graph shot.

(8) Filter window: for filter syntax refer to the software manual.

(9) Graph visualizing real-time experimental data. An individual curve can be selected by mouse click. Mouse wheel allows zooming in or out. Zoom in is also possible when selecting a rectangle area with left mouse button. For more details, see software manual.

(10) Legend panel: checked parameters are visualized in the graph.

A protocol is set of definitions that describe how to control hardware devices and accessories during an experiment. It defines the dynamics of the cultivation conditions and what, when and how is measured. Live experiment protocol can be changed even while recording.

The protocol may be as simple as having fixed cultivation parameters for the entire period of the experiment, or it may involve more elaborate settings to simulate, for example, diurnal cycles or fluctuating light/temperature environment, combined with complex measuring protocols. A protocol contains set of accessory/parameters cycle definitions. Each cycle definition can have several stages (Fig. 34-1). Each stage is defined by its duration and type (Fig. 34-2, Fig. 34-3). The stage sequence creates a cycle that is repeated automatically (cycled) until the experiment finishes.
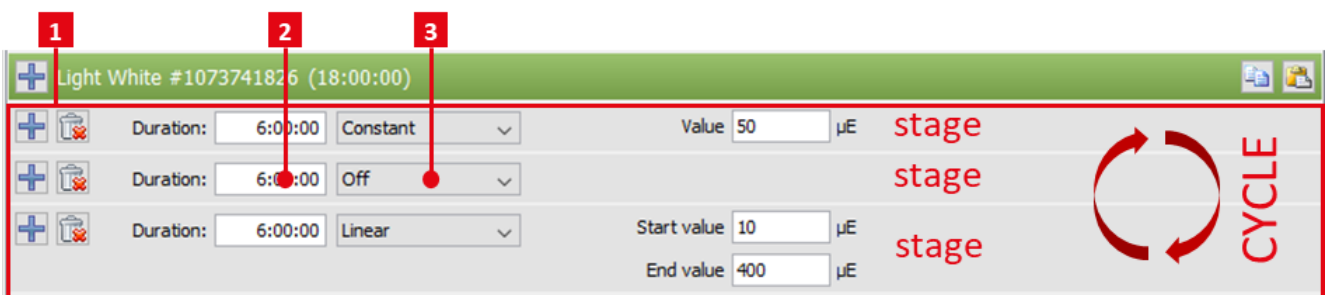

Fig. 34 PBR Control Software: Protocol definition

To define the protocol, go to tab Protocol (Fig. 35-1). Select parameters (Fig. 35-2) and define them using stages (Fig. 35-3). The parameters that were modified are labelled with M in the Protocol legend panel (Fig. 35-2). The course of the setting of the checked parameters is shown in the graph Fig. 35-4. The slider above the graph panel can zoom the graph in time axis.
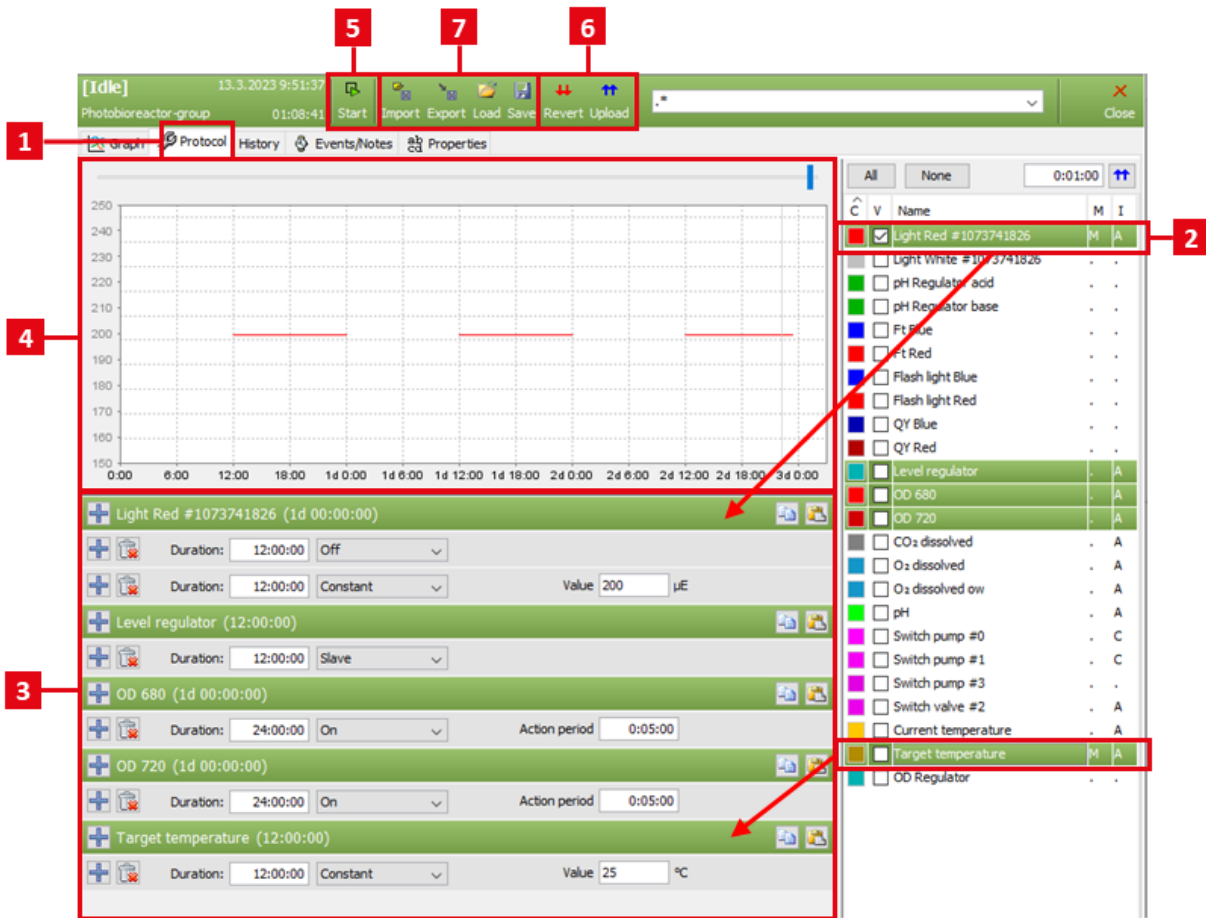
Fig. 35 PBR Control Software: Protocol panel

After the entire protocol is set, the changes must be confirmed by pressing Start or Upload button (Start button starts the experiment and at the same time, confirms the changes), Fig. 35-5, Fig. 35-6.

In the tab Protocol, the experimental protocol can be saved and loaded from the software database and also exported and imported from the local disc Fig. 35-7. The file on the local disk is accessible to the user.

	Each change in the protocol must be confirmed with the Upload button to become active.

Pumps setting

For description of the Fluidic system, please refer to the chapter Fluidic Modules. Depending on the PBR cultivation system configuration, different pump types can be linked with the PBR and set within the PBR Control Software.

Pump name	Description
Switch pump #0	Output diaphragm pump related to Turbidostatic/Chemostat/Level stat modules. If the pump is marked with C in the Protocol legend panel, it is currently automatically controlled by a regulator (Level regulator and/or OD regulator) and thus inactive in protocol. Just a flow rate of the pump can be set in the Fig. 33-4. The pump is not calibrated, its flow rate is approximate thus the pump's medium usage is just an estimated volume.
Switch pump #1	Input diaphragm pump related to Turbidostatic/Level stat modules. If the pump is marked with C in the Protocol legend panel, it is currently automatically controlled by a regulator (Level and/or OD regulator)

	and thus inactive in protocol. Just a flow rate of the pump can be set in the Fig. 33-4. The pump is not calibrated, its flow rate is approximate thus the pump's medium usage is just an estimated volume.
Switch pump #2	Bubble interruption valve. If marked with A, the pump is active and interrupts the aeration during the OD measurements. By default, the pump is turned on. It is strongly recommended to keep the default setting.
Switch pump #3	Additional possible pump.
P-Pump #1	Chemostat module, Peristaltic pump PP600, ID#1 (acid). The pump is controlled by the pH Regulator acid that has to be set in the protocol. Flow rate of the pump can be set in the Fig. 33-4. The pump can be calibrated using the external application "PumpCalibrator".
P-Pump #2	Chemostat module, Peristaltic pump PP600, ID#2 (base). The pump is controlled by the pH Regulator base that has to be set in the protocol. Flow rate of the pump can be set in the Fig. 33-4. The pump can be calibrated using the external application "PumpCalibrator".

Tab. 5 Pumps setting

⚠️	Flow rate setting of the pumps is not included in the Protocol panel thus it is not saved in the experimental protocol.

Level regulator

For a description of the Level stat, please see the chapter Fluidic Modules, for setting the Level regulator in the Protocol tab, refer to Fig. 36. The level of the medium in the vessel can be regulated in three modes that can be configured in the protocol. Each mode has several rules that define the level regulator behavior:

- High only
1. Out-flow pump is started if level is above high.
2. Out-flow pump is stopped if level is below high.

- Master
1. Out-flow pump is started if level is above high.
2. Out-flow pump is stopped if level is below high.
3. In-flow pump is started if level is below low.
4. In-flow pump is stopped if level is above low.

- Slave
1. Out-flow pump is started if level is above high.
2. Out-flow pump is stopped if level is below high or turbidostat is ON and OD is below limit.
3. In-flow pump is started if level is below low or turbidostat is ON and OD is above limit.
4. In-flow pump is stopped if level is above high or turbidostat is ON and OD is below limit

ℹ️	Hint: Select „High only" if just the output diaphragm pump is present. Select „Master" if input and output diaphragm pumps are present and the turbidostatic cultivation is not used. Select „Slave" if input and output diaphragm pumps are present and the turbidostatic cultivation is used.
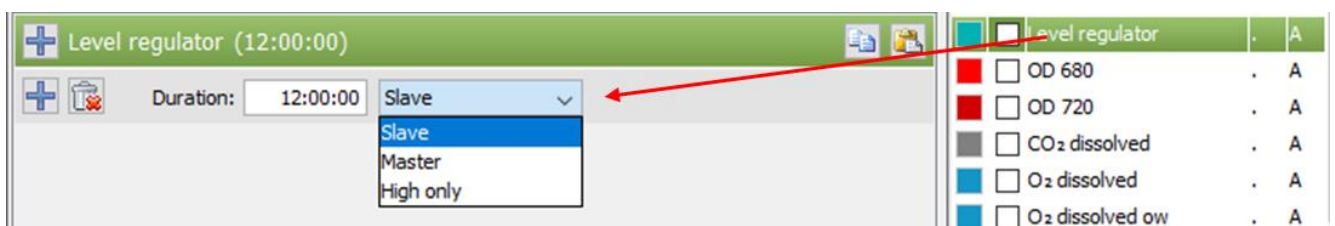
Fig. 36 PBR Control Software: Level regulator setting in Protocol panel

OD Regulator

To set a turbidostatic cultivation, select according to which OD measurement (OD 680 or OD 720) the biomass concentration will be regulated (Fig. 37-1). Furter, define the level of the requested OD, or sequence of different stages (Fig. 37-2 and 3).
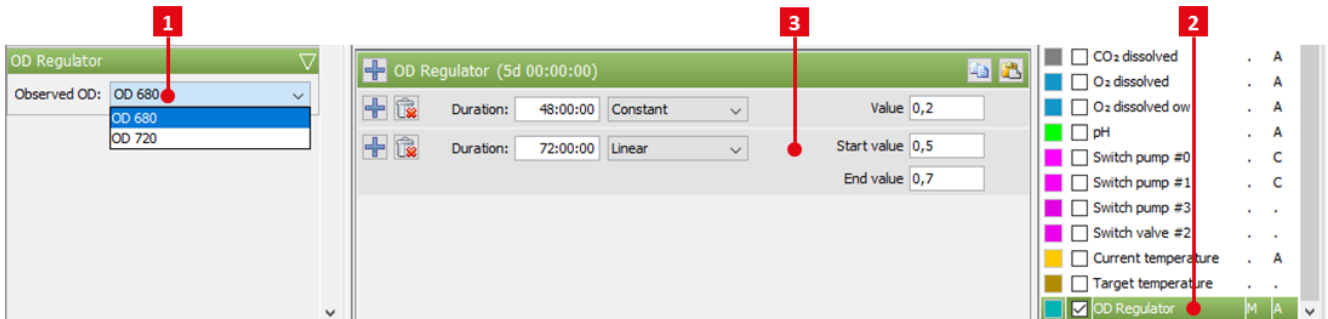

Fig. 37 PBR Control Software: OD regulator setting in Protocol panel

pH Regulators

To set a chemostatic cultivation, two pumps (P-Pump #1 and P-pump #2) and two regulators (pH Regulator acid and base) are available. For maintaining a constant pH, addition of acid is usually sufficient because the biomass pH increases with the algal concentration. An illustrative example of setting using the acid regulator is shown in Fig. 38.

Select P-Pump #1 associated with the acid solution. Check the rotation of the pump rotor and set the correct rotation in the PBR Control SW including a requested flow rate (Fig. 38-1). Choose the pH Regulator acid in the Protocol legend panel and set the requested pH value. For description of peristaltic pumps (their ID, rotation, etc.), please refer to the chapter: Fluidic Modules (description and installation).

Fig. 38 PBR Control Software: pH regulator setting in Protocol panel

8 LIST OF FIGURES