



USER MANUAL

1 L TWIN BIOREACTOR

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1. INTRODUCTION

The following user manual enables safe and efficient handling of the further listed equipment. It contains instructions, which describe the installation, operation and maintenance procedures of the 1 L laboratory bioreactor system, operation of the control unit and the SCADA (supervisory control and data acquisition) software. The user manual is an important part of the bioreactor equipment and should be kept accessible to the system operators at any time.

Before starting any work with the bioreactor system and its components, it is strongly recommended to carefully read this user manual. Full understanding of the described procedures in this document is of utmost importance for user, equipment and process safety. Only competent and trained personnel should perform operation and maintenance procedures of the bioreactor system.

In case if additional customer service or information is required, please contact your local supplier or Froilabo.

2. DESCRIPTION OF THE EQUIPMENT

The 1 L bioreactor system consists of two functional parts:

- Autoclavable **vessel**, equipped with an upper lid and inputs/outputs (ports), agitator axle with a magnetic drive, impeller turbines, baffles, gas sparger and an outlet gas condenser (equipped with a Peltier element).
- **Control unit**, equipped with a peristaltic pump system, power control unit, gas supply system, thermostat (equipped with Peltier elements) and a bottle holder.

The control unit ensures:

- 1) **Temperature** measurements and control. The temperature measurements are carried out with a Pt100 sensor. Temperature is controlled by a thermoelectric heating/cooling element (Peltier element). Thermoregulation of the reaction medium is carried out via heating/cooling the vessels bottom, which is placed in the respective temperature control platform;
- 2) **pH** measurements and control. The pH control is carried out by supplying either base or acid solutions to the bioreactors medium using the control unit's peristaltic pump. The actual pH value is monitored using a glass pH electrode. The user can pre-define the desired pH control option (for either base or acid addition);
- 3) **pO₂** measurements and control. Ensured by automatic adjustments of the stirrer's rotational speed. The actual pO₂ value is monitored using a pO₂ electrode;
- 4) **Foam** build-up indication and active control. Foam level control is carried out by supplying an antifoam agent to the bioreactor's medium using the control unit's peristaltic pump. The foam level is monitored using a conductivity sensor;
- 5) **Feeding** (of a substrate) control. Feeding is carried out using the control unit's peristaltic pump and the respective feeding rate is controlled by the feeding profile, which can be modified via the control panel;
- 6) **Mixing** control. Reaction medium agitation is ensured by a magnetically driven mixer (the stirrers shafts upper end and the motor shafts bottom end contain magnets), the agitator is driven by a motor which is mounted on the top lid of the bioreactor.

3.EQUIPMENT INSTALLATION AND ASSEMBLY

After receiving the equipment, we strongly recommend carrying out a visual inspection of the system. Inform the installation engineer or Froilabo about any visible damage of the equipment.

- 1) Install the bioreactor in a dry, free from dust and any other potential contaminants location;
- 2) The system should be installed away from any intense sources of heat. The appropriate temperature of operation for the control unit is between 5 °C and 42 °C;
- 3) Ensure a minimal space of between 15 - 20 cm at the back and on the sides of the control unit to eliminate any difficulties when installing, working and maintaining the equipment.



Warning!

To ensure your safety and safety of the system - never perform any operations with damaged parts!



Notice!

The best place for installing the equipment is one where all the necessary engineering communications (inlet/exhaust gas, power and internet) lines are accessible.

3.1 ELECTRICAL CONNECTIONS

In order to begin working with the 1 L twin bioreactor system, firstly the proper installation and connection of the operator's panel should be carried out. In order to perform the mentioned procedure, please follow the steps listed below:

- 1) Unpack the operator's panel.
- 2) Connect the **HDMI** cable, **USB** cable and display **power supply** cable jack to the operator's panel rear side (see Figure 0.1).



Figure 0.1. Operator panel connections

- 3) Place the display on top of the control unit or on the table next to the control unit.
- 4) Connect the loose ends of the mentioned cables, going from the operator's panel, to the designated connectors on the control units rear side. The **HDMI** cable should be connected to

the **HDMI** socket, the **USB** cable to the **USB** socket (see Figure 0.2). The operator's panel power supply should be connected to an external electrical outlet.



Figure 0.2. Description of rear panel connection slot placement on the control unit

- 5) Plug one end of the power cable into the control units **230VAC** socket.
- 6) Plug the other end of the power cable into an external electrical outlet.
- 7) The power toggle switch is under the label **OFF-ON**.

3.2 DESCRIPTION OF THE REACTOR LID AND PORTS

Before placing the bioreactors upper lid on the vessel make sure to install the mixers rotors in their designated places. The mentioned procedure is performed as follows:

- 1) Place the mixer rotors on the shaft in a desired location (see Figure 0.3.A).
- 2) Firmly mount the rotors in the desired position by placing the respective fastening screw in the rotors body (see Figure 0.3.B) and tightening the mentioned screw by an appropriate Allen key (see Figure 0.3.C).

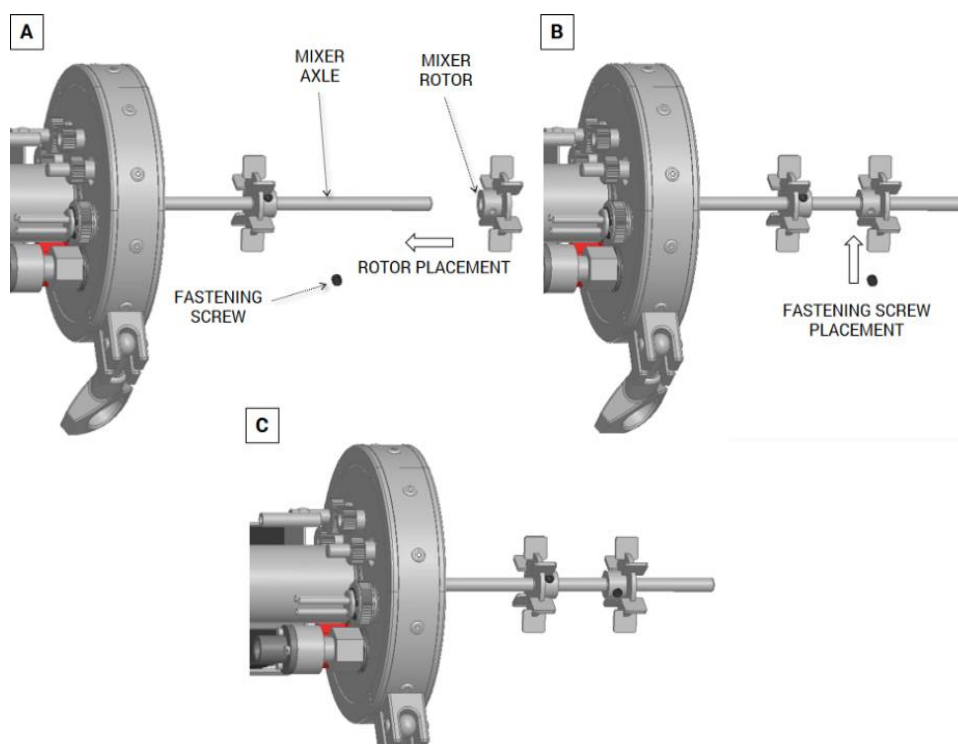


Figure 0.3. Bioreactors mixer rotor mounting schematic

The sparger installation should be performed before placing the upper lid on the bioreactors vessel. Push through the spargers upper end through the respective port and by using the supplied nut, firmly screw the sparger in place. Prior sparger mounting make sure that the respective gasket is placed between the fastening nut and the bioreactors upper lid.

The mounting procedure of the bioreactor lid on the vessel is performed as follows:

Firstly, place the **GASKET** (O-ring) between the bioreactor's upper lid and the vessel. Carefully place the upper lid clamp on the upper lid. The lid clamp has two sides (one with a teflon seal and one without a teflon seal). **Be sure to place the lid clamp so as to ensure that the teflon seal is pressing on the glass vessel flanges.** Firmly tighten the lid clamps nut, to ensure good contact between the glass vessel, O-ring and the upper lid.

After mounting the upper lid and the lid clamp, place the port O-rings in their respective places (see Figure 0.4).



Notice!

While positioning the lid gasket, use a leak-proofing lubricant (such as silicone grease).



Warning!

An improperly positioned gasket may result in a loss of sterility levels, a loss of overpressure and an efflux of gases into the environment.



Notice!

While positioning the vessel hold it by the bottom side walls.

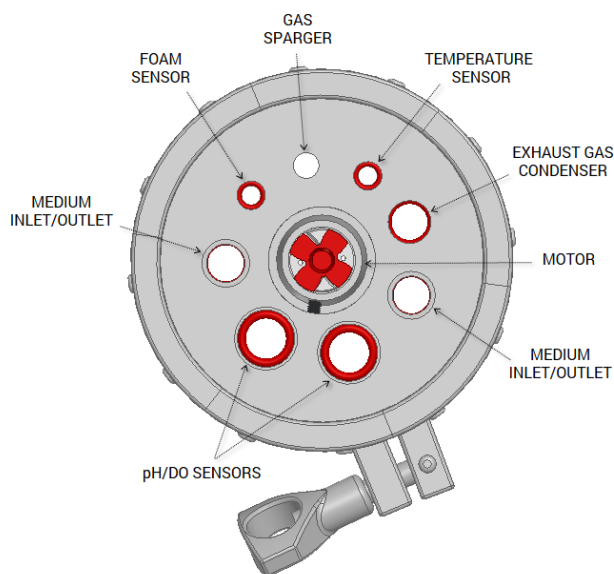


Figure 0.4. Schematic drawing showing the upper lid port layout

As a part of a standard set-up, the reactor's lid (see Figure 0.4) contains 8 ports for the following:

- Sensor installation (4 ports);
- Bioreactor's liquid supply and samples port (2 Three-way ports);
- Gas sparger and gas output ports (two ports);

For the bioreactor's liquid supply, the use of two three-way ports is envisioned (see Figure 0.5). The hemostat tube (long tube on the three-way port) is meant for medium discharge or sampling.



Warning!

When tightening the lid ports, never use a spanner. All inlets and ports must be tightened by hand. If a leak or efflux is detected, replace the respective O-rings (gasket). To ensure that no leaks occur it is recommended to lubricate the O-rings with a silicon-based food-grade grease.

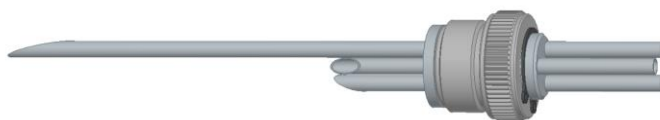


Figure 0.5. Three-way inlet port

Screw the respective three-way ports in to the bioreactors upper lid.

3.3 SENSOR AND EXHAUST TUBE INSTALLATION

To correctly and safely install sensors into the bioreactor, please follow the instructions described below.

A full bioreactor sensor system contains **four sensors**:

- **Temperature** - Pt100 temperature sensor;
- **pH** - Glass pH electrode;
- **pO₂** - Polarographic pO₂ electrode;
- **Foam** - Conductivity sensor.



Notice!

While positioning the system sensors, use a leak-proofing lubricant (such as silicone grease).

Before working with the sensors, please, carefully read the attached user manuals.



Warning!

Before inserting the sensors, ensure that the mixer is turned off! Protect the sensors from mechanical damage if they consist of or contain glass parts, polymer membranes, or other materials that can be easily mechanically damaged.

Insert the pH, Temperature, Foam and pO₂ sensors into the corresponding ports on the upper lid of the bioreactor (see Figure 0.4) and screw them in place until they are firmly attached.

The installation of the exhaust gas tube is performed similarly as in the case of sensors. Screw the exhaust gas tube in the respective port (see Figure 0.4).

3.4 INSTALLATION OF THE BIOREACTORS VESSEL

To ensure correct system functionality, each of its components must be installed in line with the instructions described further.



Warning!

Failure to comply with the instructions may cause failure in the operation of the equipment and may result in permanent damage!

The bioreactor system consists of the following main parts: (1) two stand-alone bioreactor vessels (2) control unit (3) and operators panel (see Figure 0.6).

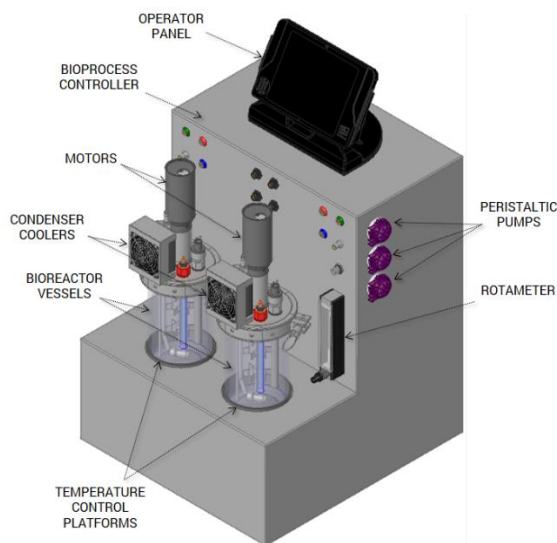


Figure 0.6. Twin bioreactor system

While installing the twin bioreactor system, the vessels should be placed on the heat transfer platforms, which are located on the control units front side as shown in Figure 0.6.

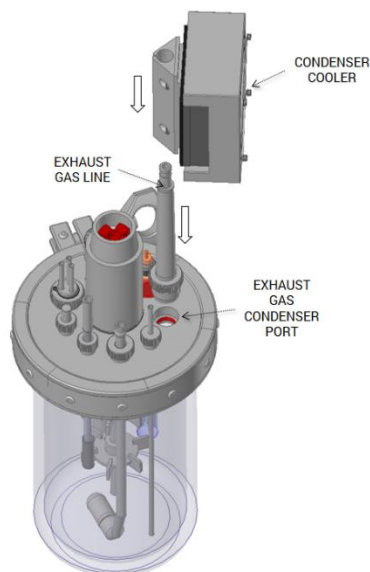


Figure 0.7. Exhaust gas tube and condenser cooler mounting

After installing the bioreactor vessels on the heat transfer platforms of the control unit, connect the respective inputs and sensors as shown in Figure 0.10.

3.5 SERVICE CONNECTIONS

The service connections for the bioreactor system can be found on the rear side of the control unit (see Figure 0.8). By using the supplied hoses and quick connections connect the air supply line, with operation parameters not exceeding the defined maximal values as shown in Table 0.1 to the designated port.

Table 0.1. Service connections

Service	Requirements	Connection type
Air	0.5 bar (max 2.0 bar)	Quick connection
Electrical power	208 - 230V, 50/60 Hz, one phase, 15 Amp (fluctuations do not exceed $\pm 10\%$)	G series plug

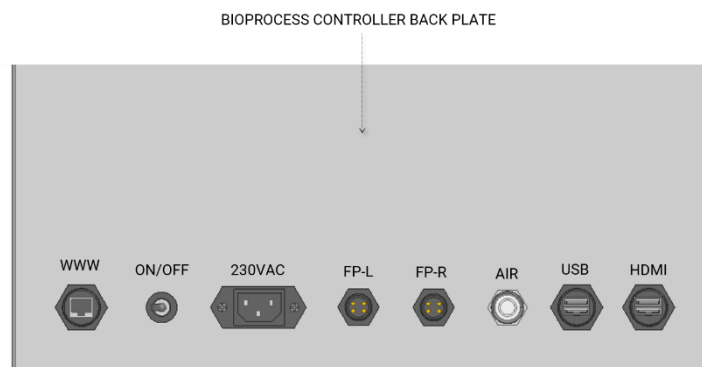


Figure 0.8. Air and oxygen service connections (back of the control unit control unit)

**Warning!**

When connecting the air supply, in order to control the pressure, the installation of pressure controllers is recommended within the permitted limits as shown in Table 0.1. For safety reasons do not exceed maximal pressure limits!

**Notice!**

When installing inlet and outlet air filters, pay attention to the filter designations. Air inlet must be connected to filter **INLET**.

3.6 VESSEL CONNECTIONS

After carrying out all of the activities listed in Section 0: "3.5 SERVICE CONNECTIONS", follow the instructions described below:

- 1) Attach the reactor inlet gas tube with an installed inlet air filter according to Figure 0.9 to the control unit's **GAS** outlet (see Figure 0.10). Connect the inlet air tube to the gas sparger port (see Figure 0.4);



Figure 0.9. Inlet/outlet gas pipes and filter set

- 7) Connect the outlet gas tube with its filter (see Figure 0.9) to the outlet air condenser at the location specified in Figure 0.4;



Warning!

Air, which is supplied to the bioreactor, should come from a clean and dry source, otherwise, water and oil particles may contaminate the filter and result in failure of the system.

- 8) Attach **T**, **pH**, **pO₂** and **FOAM** cables to the respective sensors. Then connect them to the control unit's respective ports shown in Figure 0.10;

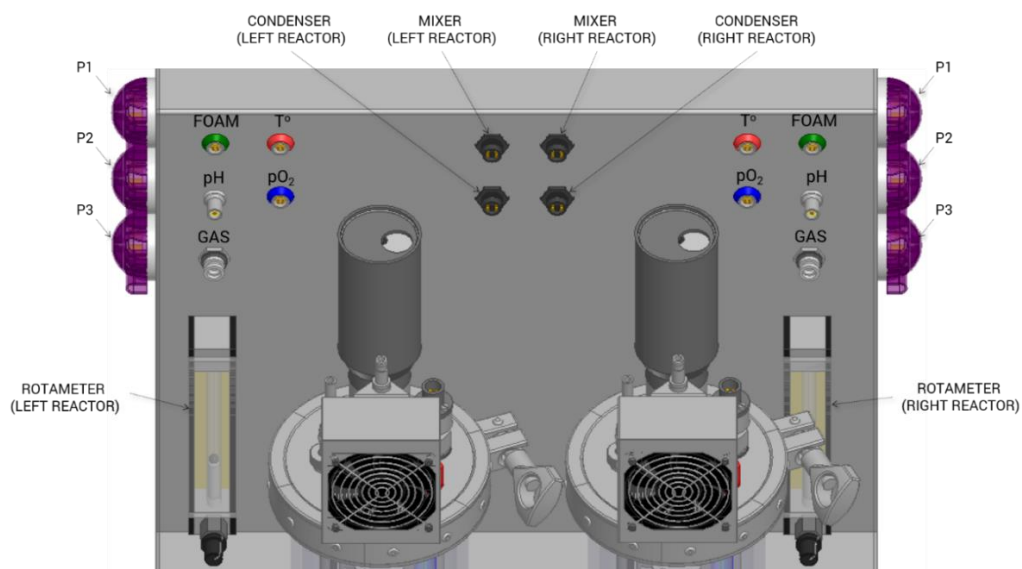


Figure 0.10. Sensor and inlet gas connection schematic

To install the **motor**, carry out the steps described below:

- 1) Turn the motor's clutch into an appropriate position, so that the magnetic couplings clutch fits inside the motors connection. Make sure to orient the motor so that the fastening screw position is aligned with the cavity of the magnetic couplings housing (see Figure 0.11.A).
- 2) Place the motor on the magnetic couplings housing (see Figure 0.11.B).
- 3) Using minimal force carefully tighten the fastening screw (see Figure 0.11.C).

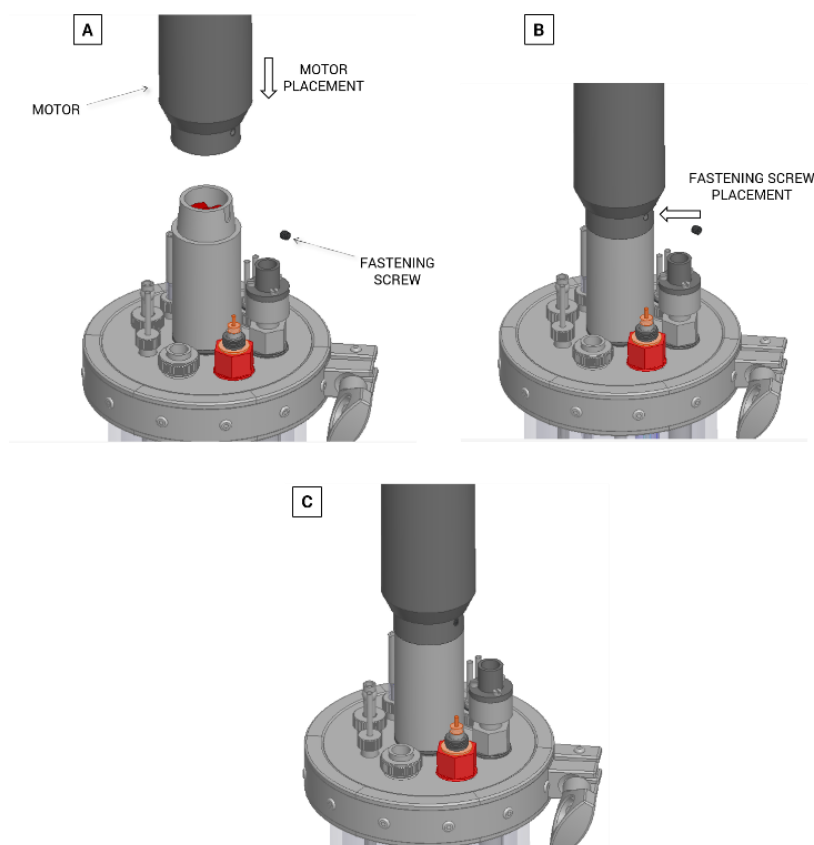


Figure 0.11. Motor installation

3.7 POWER AND INTERNET CONNECTION

In Section 3.5: 3.5 SERVICE CONNECTIONS electrical power connections for the twin bioreactor system were described. In order to start operation of the bioreactor system, perform the following connections:

- 1) Connect the electrical cable to the **POWER 230 V** input;



Warning!

Before making the electrical connections, verify whether the available voltage complies with the electrical requirements indicated on the back panel of the control unit.

Connect the unit to the electrical power only when the control unit's switch is in OFF position.

Make sure that the equipment is grounded.

In order to connect the twin bioreactor system to Internet follow the instructions listed below:

- 1) Connect the supplied MikroTik LAN router to an external power supply and switch the router ON.
- 2) Connect the router to an external Internet connection by plugging in an RJ45 cable into the socket labelled Internet PoE In.
- 3) Connect the router with the control unit by an Ethernet cable. One end of the Ethernet cable should be plugged into either the 2, 3 or 4th port of the router. The second cables end should be connected to the control units WWW port, which is located on the rear panel (see Figure 0.12).
- 4) **To control the bioreactor from a PC** it is necessary to establish a connection between the bioreactor and the PC. It can be done with a cable (see Figure 0.12), or through WiFi (see settings here below)

- a. WiFi Name (SSID) - MiniBIO_03
- b. Password – bio4bio4

Next steps are as follows

- c. Install some VNC Viewer program on the PC (for example www.realvnc.com)
- d. After launching the VNC viewer program, connect it to the bioreactor display with following settings in the viewer:
 - i. Panel IP - 172.27.3.31
 - ii. Password – 111111

When a the VNC viewer is connected, a copy of the Bioreactor display screen will appear on the PC display. Now you can control the bioreactor, using the PC mouse.

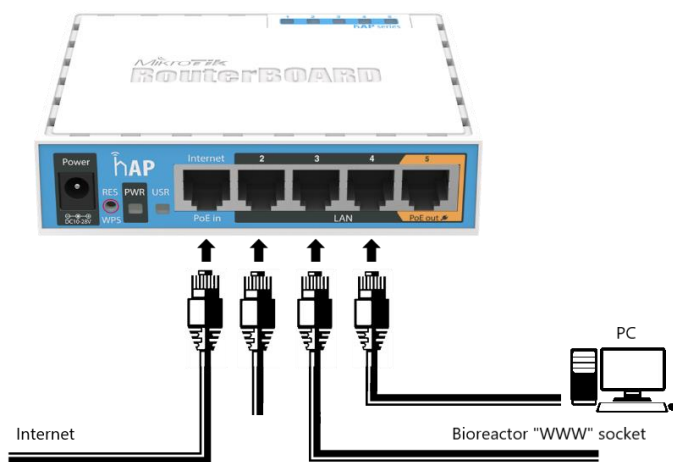


Figure 0.12. Schematic of the Ethernet connections.

Note: router port 1 is dedicated for the Internet connection.

4. SPECIFICATIONS

In Table 0.2 the main specifications of the twin bioreactor system are listed.

Table 0.2. Main specifications of the twin bioreactor system

Net weight	Control unit	~15 kg
	Vessel	~2 kg
Total dimensions		H – 510 mm; D – 430 mm, W – 460 mm
Controller		10" colour touchscreen
		Flexible software for process control
Electric power parameters		Supply 100 – 240 VAC
		Power 1000 W
Materials	Process	All process inputs, valves and related structural elements are made of 316L stainless steel (polished), 100 % boron silicate glass, polyurethane (water inputs), nylon (gas inputs - air, O ₂ , nitrogen, etc), zirconium oxide and silicon carbide ceramics, plus Teflon
	Non-process	316L or 304 type stainless steel
	Gaskets and O-rings	Rubber
Temperature	Sensor	Platinum Pt100 RTD sensor
	Control performance element	Temperature is controlled by thermoelectric heating/cooling element (Peltier element). Thermoregulation happen via heating/cooling vessel bottom inserted in copper heat transfer jar
	Type of control	PID control
	Range	At least 16 – 40 °C .
Agitation	Drive	105 W 24V BLDC motor with electronic control
	Type of control	PID control or manual settings
	Range	50 – 2000 ¹ rpm
Aeration	Air filter	0.22 µm filter sterilized by autoclaving
	Type of control	Manual and/or automated with electric magnetic valve
	Range	0 – 1 L/min
Outlet air	Air filter	0.22 µm filter sterilized by autoclaving
Peristaltic pumps		Three peristaltic pumps with configured functions (installed on the control unit)
		Option to connect the external feeding pump to an analogue control signal
Foam	Sensors	Conductivity sensor
	Control performance element	Peristaltic pump
pH (facultative)	Sensor	Sterilizable gel pH electrode
	Control performance element	Peristaltic pumps for adding base and/or acid
	Type of control	PID control
	Range	pH 2 – 12
DO (facultative)	Sensor	pO ₂ polarographic sensor
	Control performance element	pO ₂ cascade control with stirrer rotation speed
	Type of control	PID control

¹ With one mixer on shaft and aeration

5. WORKING WITH THE CONTROL UNIT

The control unit ensures control and supervision of both bioreactors.

The control unit ensures control of process parameters (T, pH and pO₂) according to their respective set-point (SP) values and the on-line logging and visualization of actual process values (PV). The operation activity and states of some control actuators (Pumps, Motors etc.) is provided.

5.1 STARTING OPERATION

To start working with the control unit follow the instructions listed below:

- 1) Turn on the controller by moving the **ON/OFF** switch at the rear side of the control unit (see Figure 0.8);
- 2) After the system has fully loaded the 1st level **REACTOR** window will appear, see Figure 0.13. More detailed information about the functionality of this window is given in Section 0: "5.2 REACTOR WINDOW";

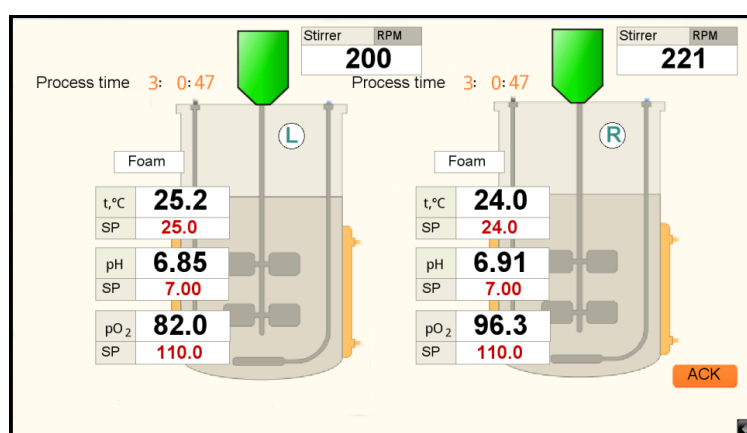


Figure 0.13. 1st level REACTOR window



Notice!

This and all of the following windows can be activated by touching the display with a finger or other object (e.g. a pencil).

- 3) By clicking on either the **LEFT** or **RIGHT** bioreactor picture the user can gain access to the 2nd level **REACTOR** window and respective setting for either the left or right bioreactor. Open the main control menu by clicking on the **MENU** button (the symbol with 3 short parallel lines, it is highlighted in Figure 0.13) inside the 2nd level **REACTOR** window. Next – press **ADMINISTRATION**, thus opening the **ADMINISTRATION** window, see Figure 0.14;

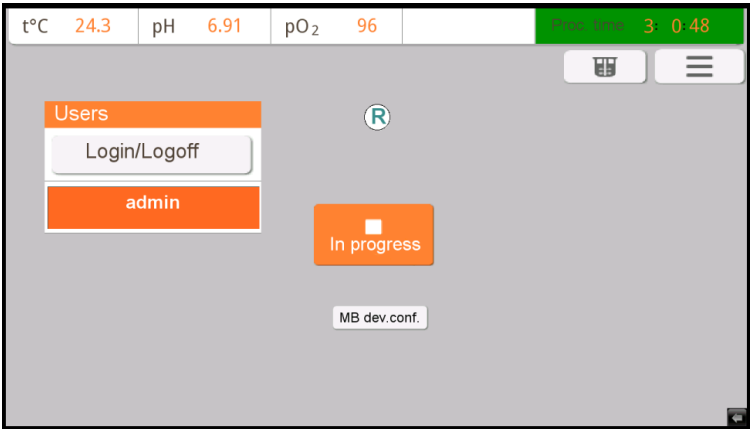


Figure 0.14. ADMINISTRATION window

- 4) After pressing the LOGIN/LOGOFF button an authorization window will appear (see Figure 0.15). Click on the blank space on the right-hand side next to the word “Password” (Figure 0.16) and using the virtual keyboard (see Figure 0.16) enter the respective password, then press **ENTER** and **LOGIN** in order to gain access to all system functions.

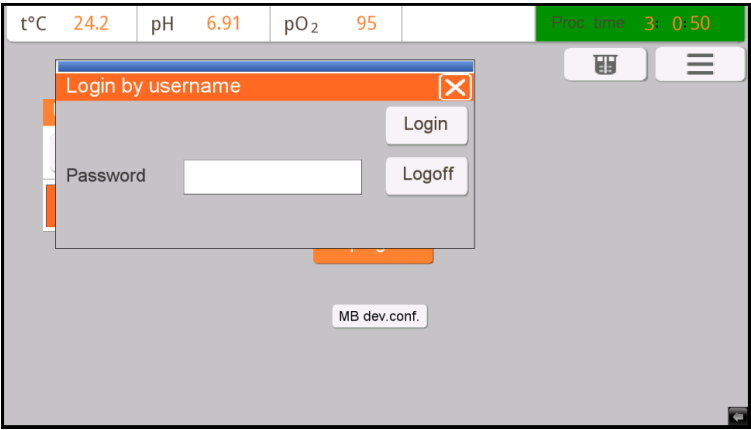


Figure 0.15. Login window

By default, the password for the above mentioned access is pre-set as follows:

Pasword
111111

The virtual keyboard allows entering desired values by pressing the respective characters and digits. Pressing **ENTER** confirms and saves the entered value. Pressing **CLEAR** deletes all entered symbols at the same time, but **BS** (backspace) – deletes symbols one by one. Pressing **Esc** (escape) allows exiting the keyboard without saving any changes.



Figure 0.16. The virtual keyboard

5.2 REACTOR WINDOW

By using the access path **MENU** → **REACTOR** open the 1st level **REACTOR** window (see Figure 0.13) for viewing both bioreactor process parameters. Alternatively, the **REACTOR** “1st level” window can be accessed by pressing the button with a schematic picture of reactor, which is placed besides the **MENU** button.

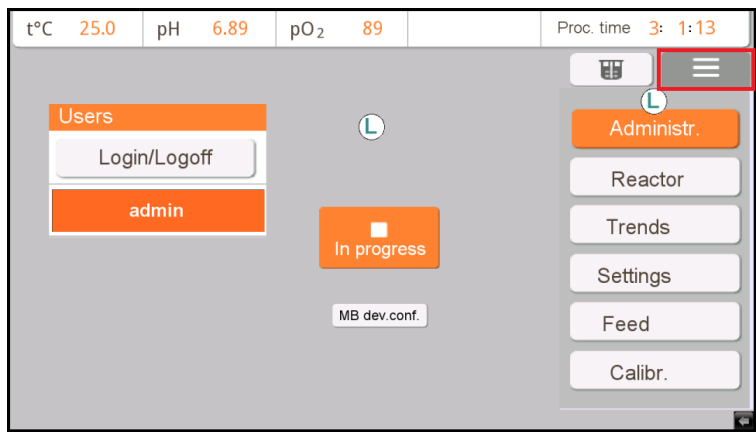


Figure 0.17. Accessing REACTOR “1st level” from menu bar

In the **REACTOR** 1st level window by pressing either the left (L) or right (R) reactor picture the user can open the respective 2nd level REACTOR windows (see Figure 0.18). In the 2nd level all of the most important process parameters can be easily configured and monitored.

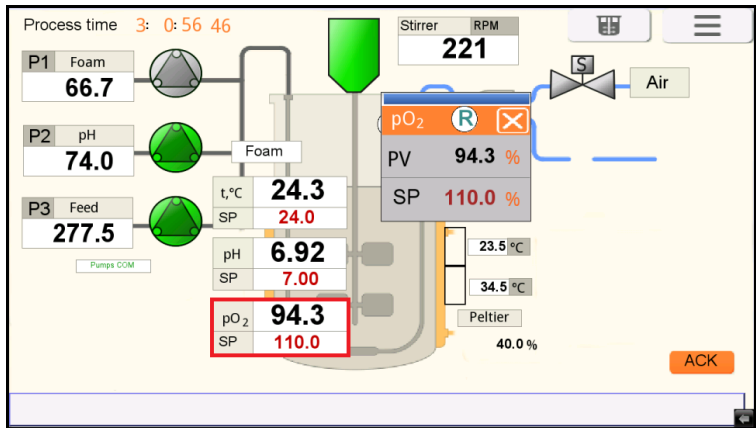


Figure 0.18. 2nd level REACTOR window

6. TEMPERATURE CONTROL

Process temperature control is carried out using thermoelectric (Peltier) cooling/heating elements located at the temperature regulation platforms.

6.1 BIOREACTOR TEMPERATURE CONTROL

To define the desired temperature set-points, using the access path: **MENU** → **REACTOR "1st level"** → **REACTOR "2nd level"**, open the **TEMPERATURE SETTING WINDOW**, highlighted in Figure 0.19.

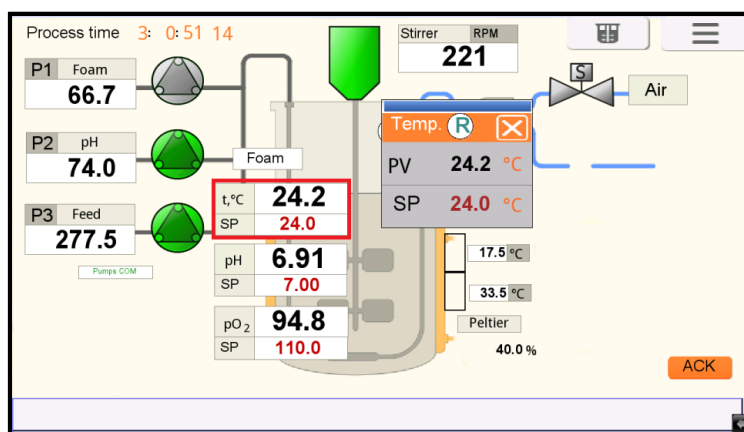


Figure 0.19. Temperature configuration window

Adjust the temperature set-points by clicking on the red digits next to the abbreviation **SP** and enter the necessary values (highlighted in Figure 0.20), confirm your action by pressing **ENTER**.

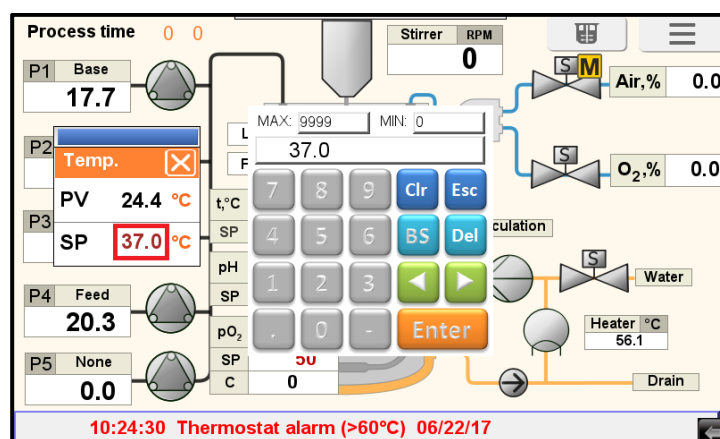


Figure 0.20. Adjusting the temperature set-points within the REACTOR window

Temperature control is carried out only in **Automatic** mode. Note that the respective Pt100 sensor connections (see Section 0: "3.6 VESSEL CONNECTIONS") should be performed prior temperature monitoring and control. Liquid in the reactor should be agitated (see Section 0: "7. AGITATION CONTROL") to maintain consistent temperature distribution. Temperature control is automatically enabled upon commencing the process.

7. AGITATION CONTROL

The mixer specifications:

- Mixer rotational speed range: 40 - 2000 rpm;
- Drive type: Magnetic;
- Impeller type: standard Rushton turbines (two pcs.).

The mixers rotational speed control can be carried out in either **Manual** or **Automatic** mode.

While in **Automatic mode** the stirrer rotational speed regulation is implemented, if the respective limits (**Min/Max**) are entered, the values are not equal to each other and the **Min** parameter is smaller than the **Max** parameter (see Figure 0.22). The stirrer rotational speed can be regulated within the range determined by the **Min** and **Max** parameters (to set them up use the following access path: **MENU** → **SETTINGS** → **LIMITS** → **STIRRER** highlighted in Figure 0.22).

While in **Manual mode** the stirrer can be switched **ON** or **OFF** manually in the **REACTOR** window by entering the respective control elements window. It is possible to pre-set a constant agitation rate while in manual mode (see Figure 0.21).

7.1 CONTROL SETTINGS

The stirrer control window can be entered by using the following access path: **MENU** → **REACTOR 2nd level** and pressing the respective symbol. In the stirrer control window it is possible to enter the stirrer's **SP** value, turn it **ON/OFF** and monitor its process value (**PV**), see Figure 0.21. Also, the desired operation mode – **Manual/Automatic** can be chosen.

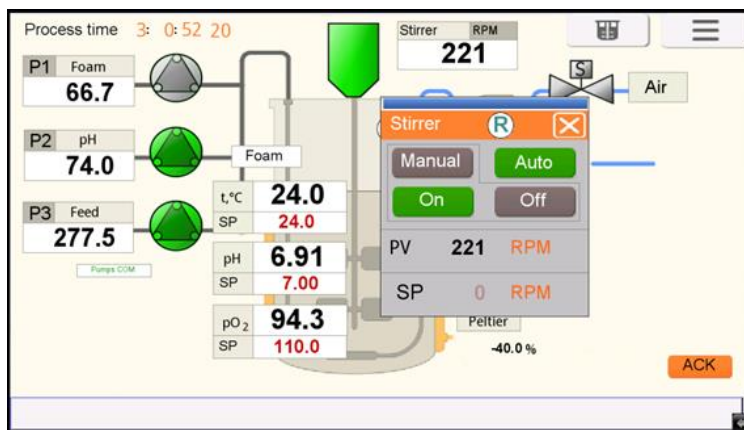


Figure 0.21. Stirrer's control window

The stirrer speed setting adjustment for pO₂ control can be performed using the path: **MENU** → **SETTINGS** → **LIMITS**.

To set-up the **Min/Max** parameters for stirrer rotational speed control, use the following access path: **MENU** → **SETTINGS** → **LIMITS** → **STIRRER** (highlighted in Figure 0.22).

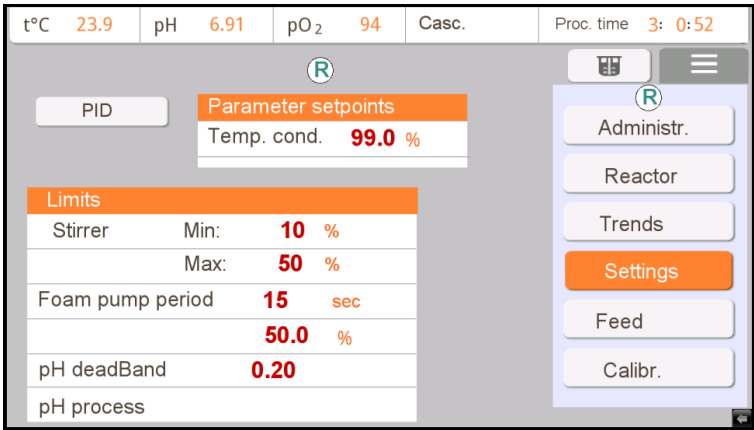


Figure 0.22. Stirrer rotational speed Min/Max parameter setting window

7.2 DISSOLVED OXYGEN (DO) CONTROL BY STIRRER ROTATION RATE

In the bioreactor system, DO concentration is controlled via automatic control of the stirrer’s rotational speed.

If the SP (Set Point) and DZ (Dead Zone) for the pO₂ value are defined, the stirrer operates in such a way, to maintain the process pO₂ value at: $SP \pm DZ$. If the pO₂ value is within the aforementioned range, the stirrer’s rotational speed remains constant. If the pO₂ value is out of this range, the agitation rate will be adjusted in respect to the PID algorithm to return the SP to the defined range. For more detailed information, see Section 0: “10. DISSOLVED OXYGEN (DO)

CONTROL”.

8. PUMP CONTROL

By default, the bioreactor system includes a 3 peristaltic pumps (see Figure 0.23) for pH control, foam control and substrate addition (feeding).

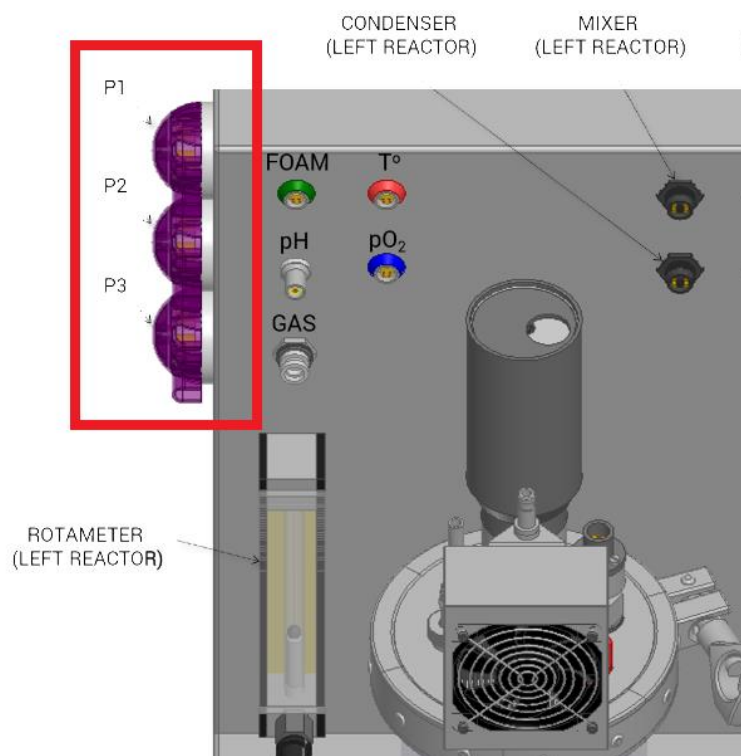


Figure 0.23. Peristaltic pumps installed

Peristaltic pumps can be operated in manual (MAN) or automatic (AUT) modes. MAN and AUT modes are described further.

8.1 TUBING

In order to mount the peristaltic pump tubes into the pump head, carry out the steps described below:

- 1) Cut the silicone tubes in a desired length. For the pH and foam control the tubes with ID=3 mm, OD=5 mm. For the feed solution addition (for fed-batch) tube with ID=1 mm, OD=3 mm are suggested.
- 2) Remove the peristaltic pump's head by turning it counter-clockwise. Place the silicone tube around the rotating part of the pumps head and place both ends into their outlets (see Figure 0.24 - A, B and C);



Notice!

The bioreactor's peristaltic pumps rotate counter-clockwise. Take this into account when placing the silicone tubes into the pump heads.

- 3) Place the pump head with an installed silicone tube back into the peristaltic pump unit by lightly pressing it in the respective socket and turning it clockwise (see Figure 0.24);
- 4) Connect one end of the silicone tube to the titrant bottle and connect the other end to the desired bioreactors port.

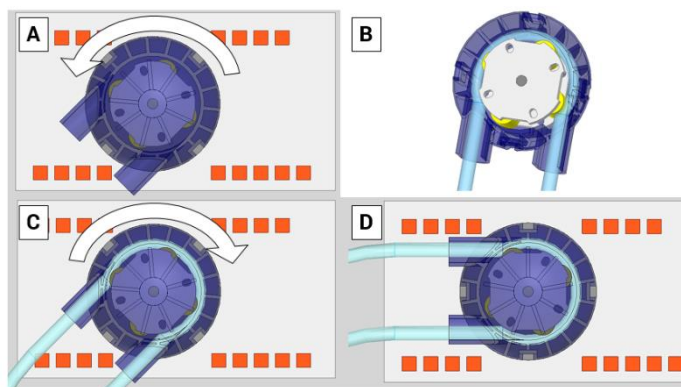


Figure 0.24. Demonstration of tube insertion into the peristaltic pump

8.2 PUMP PRODUCTIVITY

The productivity of each peristaltic pump is determined during the calibration process (see Section 0: "8.4 PUMP CALIBRATION").



Notice!

The bioreactor's peristaltic pumps minimal rotation rate is 0.6 %!

8.3 PUMP CONFIGURATION

The pumps are configured by default: Pump 1 (P1) for antifoam, Pump 2 (P2) for pH and Pump 3 (P3) for the supply of feeding solution (fed-batch procedure).

8.4 PUMP CALIBRATION

Follow the instructions listed below before performing the pump calibration:

- 1) Mount the tube, which is intended to be used into the desired peristaltic pumps head and attach the reagent bottle to the suction end of the tube;
- 2) Fill the reagent bottle with a solution that is intended to be used for pump calibration. Volume, which is pumped during the calibration procedure, should be measured by a precise measuring cylinder or scales (when using scales, keep in mind to calculate the correct volume from mass);
- 3) Access the control units 2nd level **REACTOR** window. Set the pump, which is intended for calibration (base/acid, antifoam or feed (**P1-P3**)) to automatic mode (click on the respective pump symbol and press **AUTO**);



Warning!

If the reagent bottles are not connected properly, the solution may spill while filling the pump's tube. If skin or eyes come in contact with a corrosive substance (e.g. acid, base etc.) serious chemical burns may occur. Therefore: make sure that the tube is firmly attached to the reagent bottle. When working with corrosive substances, always wear proper clothing: chemical resistant gloves, safety goggles, lab-coat etc.

- 4) Open the calibration window by using the access path **MENU** → **CALIBR**;
- 5) Use buttons **P1**, **P2** or **P3** to select the desired pump for calibration (see Figure 0.25);

The calibration for the **P1** and **P2** pump is carried out as follows:

- 1) Enter the desired calibration time (in minutes) for the **P1** and **P2** pump calibration.

- 2) Press the respective **Calibr.** button for the pump, which is meant to be calibrated. Upon pressing the said button, the pump will start working. Thus, make sure that the volume measuring cylinder or scales are empty/tared and that the exhaust end of the tube is placed inside the cylinder or a hermetic container, which is placed on the scales.
- 3) After the defined calibration time runs out the pump will switch off. Enter the liquid volume measurement, which was transferred during the calibration procedure into the **mL** field and press the **OK** button (see Figure 0.25);

The calibration for the **P3** pump is carried out as follows:

- 1) Inside the Calibr. window press the P3 button in order to access the feeding pumps calibration window. Inside the P3 pump calibration window (see Figure 0.26) enter the desired amount of calibration points (not less than 2).
- 2) For each calibration point enter the desired pump operation speed (in % from maximum) and respective calibration time (in minutes). Make sure to enter the pump operation speed in ascending order in respect to the calibration points. Do not change the values of the first calibration point, as it should be equal to zero by default.
- 3) Press the **Calibr.** button for the point, that is intended to be calibrated next and register the pumped volume of liquid.
- 4) After the pump stops enter the transferred volume of liquid into the table besides the point that was calibrated (into the **mL** field).
- 5) Press the respective **OK** button.
- 6) Repeat steps 3-5 for each calibration point.
- 7) After the calibration was carried out for all defined points press the **Validate table** button in order to check weather or not the calibration procedure was carried out correctly (the pumps productivity is proportional to the pumps operation speed).
- 8) If an error message is displayed after pressing the Validate table button, the recalibrate the points with faulty values.

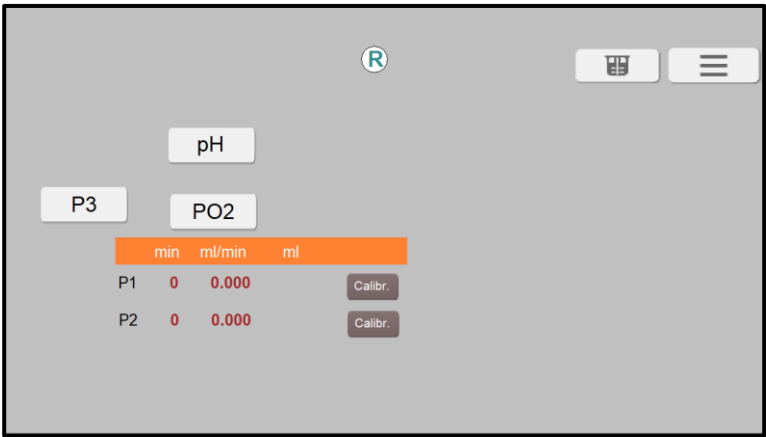


Figure 0.25. Pump 1 (P1) and pump 2 (P2) calibration window

R

	%	min	ml/min	ml		%	min	ml/min	ml	
1	0		0.000			6	100	1	2.600	Calibr.
2	1	10	0.026		Calibr.					
3	5	5	0.130		Calibr.					
4	25	4	0.650		Calibr.					
5	50	2	1.300		Calibr.					

6 Points

Validate table

ErrACK

Back

Figure 0.26. Feed pump calibration window

9. PH CONTROL

The pH value is maintained automatically at a pre-set level by means of base or acid addition to the bioreactors medium using the control unit's peristaltic pumps (see Section 0: "8. PUMP CONTROL").



Notice!

Operation and maintenance of the respective sensors has to be done according to the guidelines described in Section 0: "21.1 MAINTENANCE AND STORAGE OF THE pH SENSOR".

9.1 PROCESS PH CONTROL

To access the pH control window, open the 2nd level **REACTOR** window, locate the **pH** section (highlighted in Figure 0.1) where the **SP** value can be set.

In order to set-up the pH controls Deadband (+/- regulation value) use the following access path: **MENU → SETTINGS → pH deadBand**.

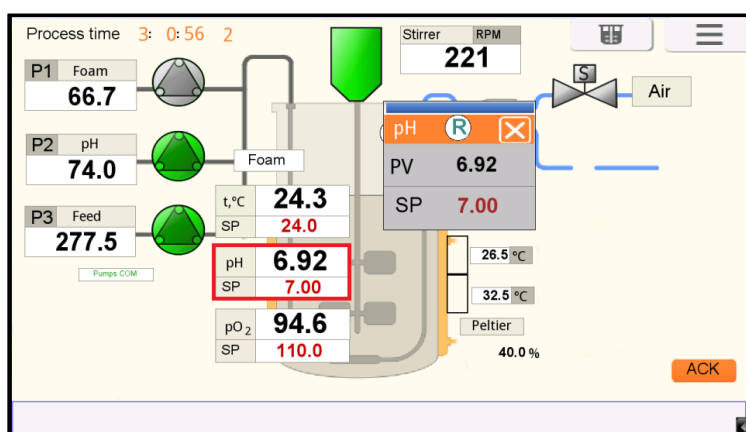


Figure 0.1. Window for setting the process pH value

pH control in the twin bioreactor system is carried out by the addition of an acidic (in case if generally the medium pH increases during the process) **OR** alkali (in case if generally the medium pH decreases during the process) solution. In order to define what type of titrant will be used for automatic pH control use the following access path: **MENU → SETTINGS → pH process**.

In order to supply an alkali solution during automatic pH control select the option: «**Acid environment**». In order to supply an acidic solution during automatic pH control select the option: «**Alkali environment**». If addition of an opposing titrant solution is needed during the process, such procedure can be performed manually, by connecting a syringe to a free end of one of the three-way needle ports.

Detailed information on process control settings, including pH control, can be found in Section 0: "16.1 CONTROLLER SETTINGS FOR STARTING THE PROCESS".

9.1 CALIBRATION OF THE pH SENSOR

For measurement reliability during the fermentation process, it is strongly recommended to calibrate the pH sensor before each process. Before performing the calibration procedure, two buffer solutions should be prepared.



Warning!

To avoid any inaccuracies during calibration, keep in mind that the pH sensor must be rinsed with distilled water before submerging it into the buffer solution or before storage.



Warning!

Do not touch or wipe the sensors end.



Notice!

If any signs of sensor improper work are suspected (e.g. increased response time, increased membrane resistance, exceeding of maximum sterilization cycle amount (20 - 50)) it is recommended to check the pH sensor calibration accuracy after the sterilization process by using a reference electrode.

By using the access path **MENU** → **CALIBR.** → **pH**, open the pH calibration wizard (see Figure 0.2);

Figure 0.2. pH Calibration wizard

In order to calibrate the pH sensor, follow the instructions outlined further:

- 1) In order to start the calibration procedure press the **Start** button.
- 2) Enter the buffer solution temperature in the pH calibration window by clicking on the digits (which are indicated under the title "Temperature");
- 3) Enter the first buffer solution pH value (see Figure 0.3) by clicking on the digits under the **1. POINT** button.



Notice!

In the bioreactor system, pH compensation according to the measured buffer temperature is maintained using the temperature sensor signal. Therefore, for precise pH measurements simultaneously measure the actual buffer solution temperature using the bioreactor's temperature sensor.

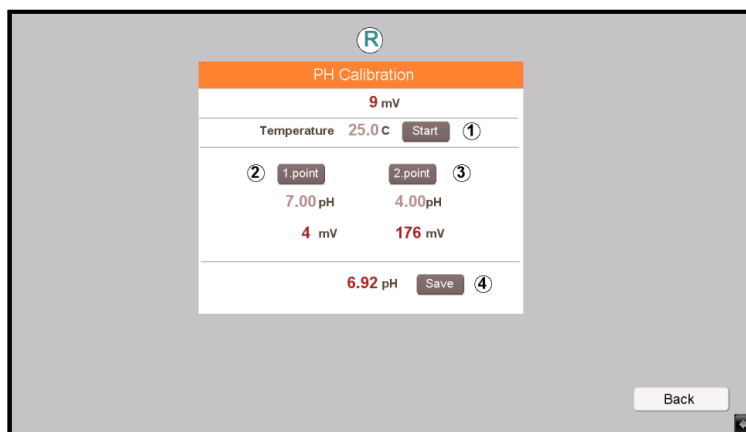


Figure 0.3. pH calibration wizard for calibration of the first buffer solution

- 4) Immerse the sensor into the buffer solution, and wait until the mV readings stabilize;
- 5) When the mV value has stabilized – press the **1. POINT** button.
- 6) Enter the second buffer solution pH value by clicking on the digits under the **2. POINT** button.
- 7) Immerse the sensor into the second buffer solution, and wait until the mV readings stabilize. When the mV value has stabilized click the **2. POINT** button.
- 8) After the calibration procedure has been carried out press the **SAVE** button.
- 9) Exit the pH Calibration wizard by clicking the **BACK** button.



Warning!

For long time storage, the sensor should be placed in a saturated KCl solution and left affixed to a stand (the sensor membrane should be fully submerged in the solution and the container should be sealed in order to reduce the solution evaporation).

10. DISSOLVED OXYGEN (DO) CONTROL

The dissolved oxygen (DO) level in the culture medium is measured as dissolved oxygen partial pressure (pO_2). pO_2 is expressed in % from maximally possible oxygen concentration that is determined during sensor's second point (maximal oxygen saturation (100 %)) calibration. In the twin bioreactor system, DO can be controlled via automatic mixer speed setting adjustment.



Notice!

Handling of the pO_2 sensor has to be done according to the instructions described in Section 0: "21.2 MAINTENANCE AND STORAGE OF THE pO_2 SENSOR".

To access the pO_2 control window, open the 2nd level **REACTOR** window, locate the pO_2 section (highlighted in Figure 0.4) where the **SP** value can be set.

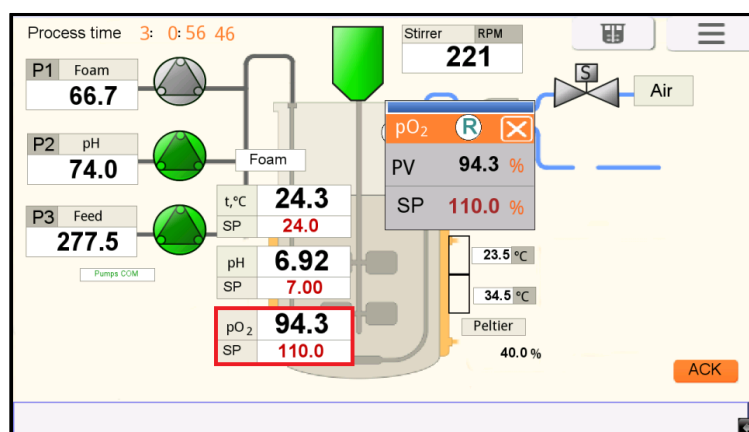


Figure 0.4. Window for setting the processes pO_2 value

Detailed information on process control setting selection, including pO_2 control setting selection, is listed in Section 0: "16.1 CONTROLLER SETTINGS FOR STARTING THE PROCESS".

10.1 GENERAL INFORMATION ABOUT THE pO_2 SENSOR'S CALIBRATION

In general, pO_2 sensor's calibration procedure should be carried out after sterilization. The test calibration prior sterilization is advised for inspection of the sensor and system's operating conditions.

The calibration procedure can be performed by **two point** linearization. For calibration the sensor has to be subjected to either oxygen saturated and/or oxygen-free environments.



Notice!

During zero point (0 %) calibration it is possible to evaluate the sensor operating conditions and potential maintenance requirements. Information about the possible malfunctions and their causes can be found in the supplied sensor user manual.



Notice!

Prior calibration, polarographic pO_2 sensors require a time period of at least 6 hours to polarize. Note that in case if you are using a different type of sensor, reference to the sensor user manual about important information on handling and maintaining the equipment.

Ensuring **oxygen-saturated** environment:

- Sensor exposure to air. Affix a clean and dry sensor to a stand or in an aerated bioreactor's tank without any liquid. This method can be used before and after sensor sterilization.
- Sensor exposure to agitated and aerated bioreactor environment. This method is best suited for simulation of oxygen-saturated environment in conditions, which most closely reproduce the maximal oxygen concentration, which can be achieved within the fermentation process. Distilled water, culture medium or fermentation broth can be used as a liquid phase. Maintain the liquid temperature and vessel's inner pressure at values as close as possible to those, which are to be used during the fermentation process. **Agitation and aeration rates should be set-up as maximal limits used in the process, taking into account the culture tolerance to mechanical damage!** Typically, stirrer's rotational speed of 800 rpm and 0,2 – 0,4 slpm of aeration can be applied if the process will run using shear stress resistant microorganism (e.g. bacteria, yeast etc.). This method can be used before and after sensor sterilization.

Ensuring **oxygen-free** environment:

- Sensor exposure to a saturated Na_2SO_3 water solution. It is possible to simulate an oxygen-free environment using a saturated water solution of Na_2SO_3 . It is recommended to use a fresh solution during every zero point calibration procedure to make sure that the oxygen is fully absent in the liquid.
- Sensor exposure to the bioreactor environment aerated with nitrogen (N_2) gas. Distilled water, culture medium or fermentation broth can be used as a liquid phase. Typically, stirrer rotation speed of 800 rpm and 0,2 – 0,4 slpm of nitrogen gas can be applied. Under these conditions, oxygen displacement normally happens within 5 – 10 min. This method can be used before and after sensor sterilization.
- Sensor exposure to nitrogen (N_2) gas. Affix the sensor in a partially enclosed vessel and introduce nitrogen (N_2) gas into the vessel.



Notice!

We recommend registering every calibration procedure results, such as nA values and time of stabilization during each point calibration (0 % or 100 %). This allows evaluating the measurements reliability and helps preventing sensor malfunctions during the fermentation process. Information about the required maintenance procedures can be found in the sensor user manual.

10.2 pO₂ SENSOR'S CALIBRATION

0 % point calibration

Choose one of the methods described in Section 0: "10.1 GENERAL INFORMATION ABOUT THE pO₂ SENSOR'S CALIBRATION" – "Ensuring **oxygen-free** environment" and follow the instructions listed below to carry out the pO₂ sensor calibration.

- 1) Use the access path **MENU** → **CALIBR.** → **pO₂**, to open the pO₂ calibration wizard (see Figure 0.5)

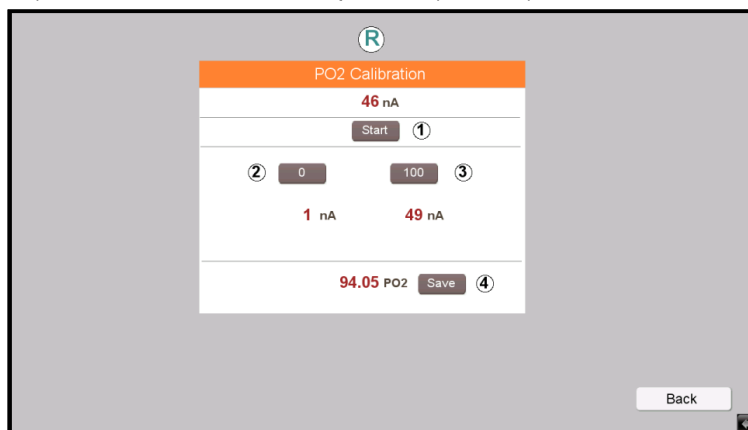


Figure 0.5. pO₂ calibration wizard

- 2) Press the **START** button in order to commence the calibration procedure.
- 3) Subject the electrode to an oxygen-free environment and wait for the nA reading to stabilize (usually in the range of 0 – 1.5 nA),
- 4) Press the **0** button.

100 % point calibration

Choose one of the methods described in Section 0: "10.1 GENERAL INFORMATION ABOUT THE pO₂ SENSOR'S CALIBRATION" – "Ensuring **oxygen-saturated** environment" and follow the instructions listed below to carry out the pO₂ sensor calibration.

- 1) Subject the electrode to an oxygen-saturated environment and wait for the nA reading to stabilize (usually in the range of 30 – 50 nA);
- 2) Press the **100** button.
- 3) After finishing the calibration procedure press the **SAVE** button.
- 4) Exit the pH Calibration wizard by clicking **BACK**.

11. FOAM CONTROL

Foam regulation can be carried out by supplying an anti-foam agent to the bioreactor's environment using one of the bioreactor's peristaltic pumps. Control is implemented using the respective conductivity sensor, which supplies a signal to the control unit when the foam rises to a pre-set level. If the sensor signal becomes active; within the 1st or 2nd **REACTOR** window the **Foam** alarm is signalized. After the control unit receives the sensor signal, the respective pump starts operating and pumps antifoam to the bioreactor environment according to the pre-set settings until the signal becomes inactive.

11.1 FOAM CONTROL PRINCIPLE

The foam sensor should be installed according to the peculiarities of each specific process. The installation includes configuration of the distance (the optimum distance is around 1 – 3 cm) between the fermentation environment and the lower part of the foam sensor. The antifoam pump operation conditions can be adjusted using the access path **MENU → SETTINGS → FOAM PUMP PERIOD** (see Figure 0.1).

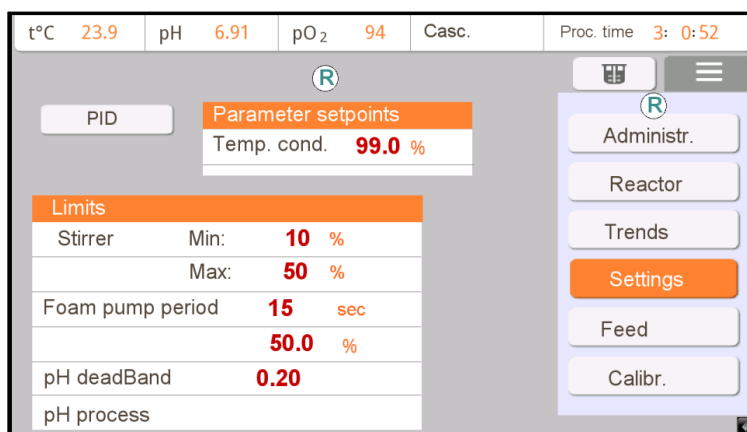


Figure 0.1. Foam pump period settings

The Foam pump period value defined the period of operation of the peristaltic pump if a foam level signal is received. The % value correspond to the ON state of the pump during the Foam pump period (which was described earlier). According to the information presented in Figure 11.1, the antifoam supply pump will operate for 7.5 seconds (50% from 15 sec.) each 15 sec. if a foam level signal is received.

Detailed information on process control setting selection, including foam control setting selection, can be found in Section 0: "16.1 CONTROLLER SETTINGS FOR STARTING THE PROCESS".

12. PROCESS TRENDS

To open the trends menu use the access path **MENU** → **TRENDS**, see Figure 0.2.

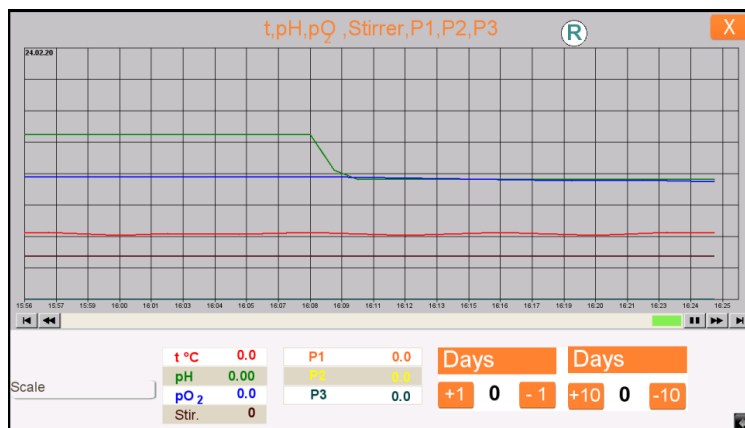


Figure 0.2. TRENDS window

The **TRENDS** window displays the changes in the dynamics of process parameters (temperature, pH, pO₂, stirrer, pumps, etc.), by clicking on buttons **1** and **2** the user can look-up trends of other parameters.

By clicking on **◀◀** or **▶▶** it is possible to move trends backwards or forwards, and clicking on **◀** moves the trends to the start of the process, but **▶** moves trends to the current minute, if the process is still going or to the end of the process if it has already finished.

By clicking on the **MIN - MAX** button, it is possible to adjust every individual parameter **Y** axle's minimal and maximal values, which are displayed in the **TRENDS** window.

13. SETTING UP PID PARAMETERS

Open the PID settings window by using the access path **MENU** → **SETTINGS** → **PID**, highlighted in Figure 0.3.

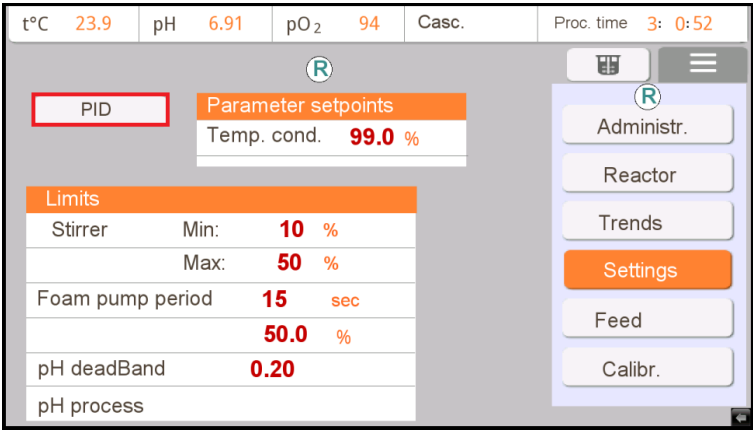


Figure 0.3. SETTINGS window (PID)

PID parameter coefficients for pH and pO₂ control can be modified in the PID configuration window (see Figure 0.4).



Warning!

Default PID parameter coefficients are pre-set by the supplier. Only trained and advanced personnel should modify these parameters, because they significantly influence the performance system controls. Change them at your own risk. If additional PID parameter configuration information is required, contact *Froilabo* or your local supplier.

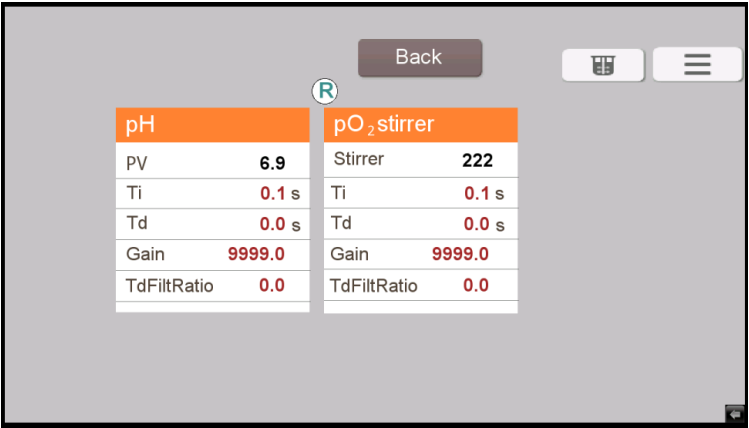


Figure 0.4. Window for setting up pH and pO₂ control PID parameters

14. FEED CONTROL

Bioreactor system ensures feeding control by a time profile.

14.1 TIME PROFILE OF FEEDING RATE

In the twin bioreactor system the possibility to control feeding by means of adjustable profile has been envisioned. The following section describes the principles of working with the profile.

The feeding profile window can be opened using the following access path: **MENU** → **FEED**.

t°C24.2

pH6.92

pO₂94

Proc. time3: 0:58

R

Stop feed

Clear table

N ₂	Min	ml/min	N ₂	Min	ml/min
1.	0	0.00	11.	300	1.50
2.	30	0.00	12.	390	1.00
3.	60	0.50	13.	450	0.50
4.	90	0.50	14.	480	0.00
5.	120	0.00	15.	740	0.00
6.	150	0.50	16.	800	0.00
7.	180	0.50	17.	860	0.00
8.	210	1.00	18.	980	0.00
9.	240	0.00	19.	1100	0.00
10.	270	0.00	20.	1220	0.00

Figure 0.5. FEED window, TIME SHIFT pop-up window

Within the **FEED** window (Figure 0.5) it is possible to set-up 20 values of time and corresponding feeding rates. Time tags correspond to the fermentation process time.

For correct operation of the system the first point in the feeding profile has to be equal to zero (both time and productivity) at all times.

Each feeding profiles point is defined by time (in minutes) – calculated from the moment the feeding is activated and feeding solution supply productivity (in mL/min). After the feeding is enabled, the feeding supply productivity is corrected each minute according to a linear approximation between two adjacent points within the feeding profile.

During the process it is possible to adjust the feeding profile. Corrections will be taken into account on actual (running) feeding profile points, beginning of the next minute.

Feeding is activated by pressing the **START FEED** button. Feeding can be stopped using the same button that indicates **STOP FEED** while the feeding profile is active.

15. PREPARATION FOR THE PROCESS

Before enabling the process, always carry out the activities listed below and inspect the respective system elements:

- 1) Check the operational conditions of the system's software, bioreactor's vessel, its constituents and other equipment;
- 2) Make sure that the bioreactor vessel is located correctly in respect to respective service connections;
- 3) Make sure that **all service connections** have been carried out according to Section 0: "3.5 SERVICE CONNECTIONS";
- 4) Carry out the **calibration of sensors and pumps** which are to be used during the process (information about calibration of each device is provided in the upper Sections of this user manual, see the table of contents);
- 5) Make sure that the **ports and their connections have been placed, screwed, connected, etc. correctly** (more detailed instructions for the respective activities can be found in Section 0: "3.2 DESCRIPTION OF THE REACTOR LID AND PORTS", Section 0: "3.3 SENSOR AND EXHAUST TUBE INSTALLATION" and Section 0: "3.6 VESSEL CONNECTIONS";
- 7) Carry out **manual** (in manual mode) **inspection of the work of every control element**, which is intended to be used during the process. It is necessary to ensure flawless operation of the system; therefore, potential errors/inconsistencies should be detected and solved;
- 8) Carry out **sterilization of the bioreactor tank and its inputs** (which will be in contact with the reaction environment) as described in Section 0: "20. STERILIZATION OF THE VESSEL";
- 9) Repeat steps 1-3, 5 and 6 after the sterilization of the bioreactor vessel and its inputs! Make sure that the reactor's tank is liquid-free (if it was not sterilized with the reaction environment or other liquids), and that the sampling port is **closed**.



Warning!

Inappropriate equipment preparation for work may lead to serious physical injury or equipment damages. To avoid the upward mentioned, following instructions in this user manual, are highly recommended.

15.1 ADDING THE REACTION ENVIRONMENT TO THE BIOREACTOR VESSEL

When all activities listed in Section 0: "15. PREPARATION FOR THE PROCESS" have been carried out, the reaction environment can be supplied to the sterilized bioreactor vessel. If the bioreactors vessel was autoclaved with water, then prior reaction medium addition the water should

be pumped out. The mentioned procedure is described in Section 17: "17.

SAMPLING AND HARVESTING".

To supply the reaction environment to the vessel, follow the instructions described further:

- 1) Connect the previously prepared vessel with sterile reaction environment to one of the needle ports or the chemostat tube, installed on the upper lid of the bioreactor, via a sterile hose;
- 2) By using the feeding pump (see Section 0: "8. PUMP CONTROL") carrying out manual addition of the reaction environment to the vessel;
- 3) After reaction environment has been supplied, stop the pump, remove the previously connected tube and close the bioreactor port with a previously prepared sterile cap/plug.



Notice!

All connections should be performed in sterile conditions (e.g. in the presence of flame, laminar flow cabinet, etc.).

16. STARTING THE PROCESS

Before enabling the process using the system, instructions described in Section 0: "15. PREPARATION FOR THE **PROCESS**" should be adapted as was previously described.

16.1 CONTROLLER SETTINGS FOR STARTING THE PROCESS

Before starting the process, carry out the following actions to set-up the control unit settings:

- 1) Set the **control parameter values (SP)** from the 2nd level **REACTOR** window and change the set-points there for every desired control parameter;
- 2) Set the limits of **STIRRER** rotation speed by using the access path: **MENU** → **SETTINGS** → **STIRRER (Min/Max)**.
- 3) Set the antifoam supply pumps settings by using the access path: **MENU** → **SETTINGS** → **Foam pump period**.
- 4) Set the pH control pumps settings by using the access path: **MENU** → **SETTINGS** → **pH deadBand/pH process**.
- 5) Set the desired **control elements** to **automatic mode**. In the **REACTOR** window, the respective parameters, which are to be controlled automatically, have to be set to **Auto**;

The bioreactor system is ready for the process running!

16.2 INOCULATION

It is recommended to carry out inoculation, using one of the three methods described below: (1) inject the microorganism culture into the reaction environment prior supplying it to the bioreactor tank, (2) using a peristaltic pump transfer the inoculum to the vessel after supplying the reaction environment to the bioreactors tank or (3) inject it into the bioreactor through a free port. In all cases the activities have to be carried out in line with the instructions described in Section 0: "

15.1 ADDING THE REACTION ENVIRONMENT TO THE BIOREACTOR VESSEL".

To eliminate the process lag phase and to avoid improper fermentation conditions, it is recommended to inoculate the culture into the reaction environment with a temperature, that is close to the seed material and/or the processes temperature. The temperature can be maintained by adding the reaction environment with a desirable temperature to the bioreactor, or by automatic temperature control of environment inside the bioreactor (see Section 0: "6.1 BIOREACTOR TEMPERATURE CONTROL").



Warning!

Regardless of the chosen inoculation method, culture inoculation in the temperature and pH conditions far from desirable set points can irreversibly harm the culture.

16.3 STARTING THE PROCESS

To enable the process click the **START** button in the **ADMINISTRATOR** window (see Figure 0.6).

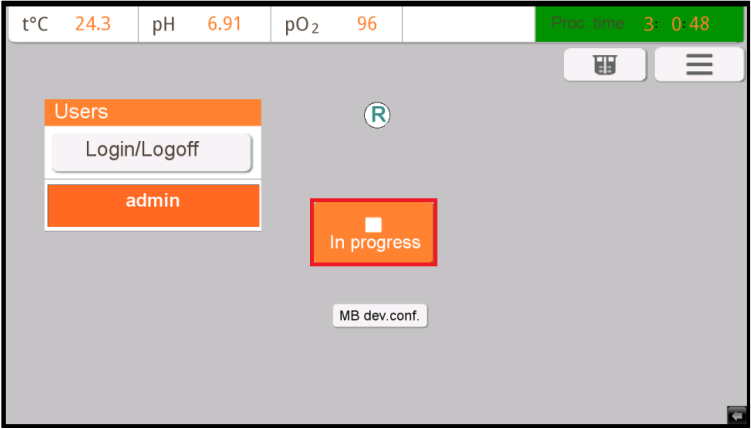


Figure 0.6. Window for starting the process

17. SAMPLING AND HARVESTING

The method of culture sampling and harvesting can vary depending on user requirements and fermentation process specifics. The suggested sampling and harvesting procedures for the twin bioreactor system are described below.

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Both sampling and harvesting actions can be performed through the reactor's lid using a chemostat tube.

The sampling volume can vary depending on:

- The analytical measurement requirements;
- The amount of the culture or product in the vessel;
- The duration of the fermentation process;
- Etc.

Typical sample volumes for the bioreactor system are 0.1 – 1 mL.

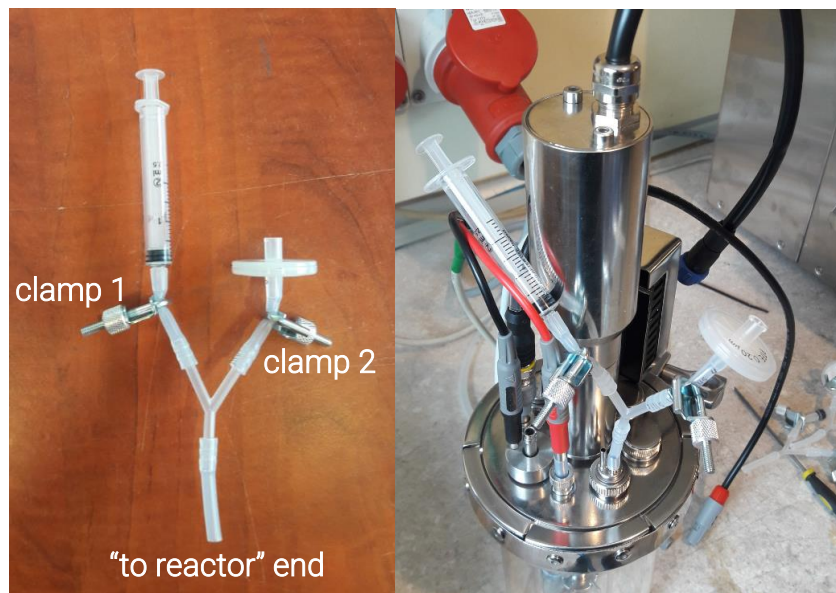


Figure 0.7. Sampler overview

Sampler attachment and sampling is performed as follows:

- 1) The sampler (Figure 0.7, left side) **"to reactor"** end should be attached on the reactor's chemostat tube port (long tube immersed in culture);
- 2) Close the **clamp 2** and open **clamp 1** (Figure 0.7, left side);
- 3) Gently pull out the syringe piston to suck the appropriate amount of sample volume;
- 4) Close the clamp 1 and open clamp 2 to allow culture remaining in sampler peripheral to flow back to reactor;
- 5) Disconnect the syringe for sample further processing; While disconnecting the syringe, to avoid vessel contamination, follow the aseptic procedures (like disconnections in the sterile zone of the flame, avoid long syringe- silicone tube disconnection periods etc.).
- 6) If the **calmp 1** are located maximally close to syringe end, then no significant amount of culture dead volume should stay in connection syringe-silicone tube, otherwise spill out or wash out with the alcohol collected culture dead volume.



Notice!

To avoid culture contamination sterilize the sampler together with vessel.

17.1 HARVESTING

Culture harvesting possible utilizing chemostat-tube-syringe system. In this way, use the peristaltic pump for culture harvesting from the vessel. Also harvesting possible by dismounting the reactor lid and by direct pouring the culture in the appropriate vessel.

18. COMPLETION OF THE PROCESS

To stop the fermentation process, use the same access path as used when enabling the process:
MENU → ADMINISTRATION → STOP PROCESS.

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For safety purposes, control elements (cooling valve, heater, stirrer, pumps, air valve and O₂ valve) should be switched to **Manual** mode after the process has been stopped.



Warning!

Turning the control elements to Manual mode after the process run, minimizes the risk of unplanned system operation and its damage upon accidental launch of process simulation!

19. CLEANING OF THE VESSEL

After the process has been stopped, disconnect the bioreactor vessel from all service communications.

Carefully remove sensors from the tank. Follow instructions listed in Section 0: "21.

MAINTENANCE", to ensure appropriate maintenance and storage of the pH and pO₂ sensors.

Thoroughly wash and clean the bioreactor vessel and the corresponding parts (ports, sensors, etc.).

Unscrew the outlet air condenser according to the instructions outlined in Section 3.3: "3.3 SENSOR AND EXHAUST TUBE INSTALLATION".

Bioreactor turbine removal and cleaning is highly recommended after every fermentation process, see instructions on Section 0: "

3.2 DESCRIPTION OF THE REACTOR LID AND PORTS".

20. STERILIZATION OF THE VESSEL

To sterilize the vessel in the autoclave, follow the instructions described below:

- 1) Install all necessary sensors and other accessories in the bioreactor's tank, taking into account that the bioreactor has to be hermetical during the sterilization procedure;
- 2) Fill the vessel with distilled water or culture media if high temperatures and pressure will not affect its chemical composition;



Warning!

During vessel sterilization it is highly recommended to prevent the sensors from being dry through the procedure, therefore, fill the bioreactor with liquid in order to avoid any possible sensor damage.

- 3) Disconnect the motor and all sensor cables;



Warning!

Never put any electrical cables into the autoclave for sterilization, this can cause irreversible damage! **Do not forget to dismount the cooler of the outlet gas condenser!**

- 4) Disconnect all silicone tubes from the reagent bottles. Close the tube ends by putting clamps on them - as close to the tube ends as possible;



Notice!

Tubes have to be clamped in order to prevent any liquid efflux from the bioreactor vessel during sterilization.



Warning!

During sterilization tubes can burst or slip off the inlet ports due to overpressure or damaged tubes.

Therefore – sterilize only tubes that are intact, thoroughly rinsed - never sterilize tubes if they are filled with corrective reagents!

- 5) Place the bioreactor vessel into the autoclave;
- 6) Carry out the vessel sterilization (120 °C for 45 min is recommended);
- 7) When the sterilization procedure has been finished and the vessel has cooled down, take it out of the autoclave and place it in its planned working location;
- 8) Carry out required operations, which are to be made with the bioreactor.



Warning!

In order to avoid severe skin burns due to the high temperature:

- Always wear protective clothes and heat resistant gloves while working with the autoclave or any other hot equipment.
- Ensure that all devices are cooled down to room temperature before use.
- It is recommended to place a suitable warning notice e.g. "Hot".

21. MAINTENANCE

In the following sections the maintenance and preparation procedures for the pO_2 and pH sensors are described. Keep in mind that the information in this user manual should be comprehended together with the information supplied by the sensor's manufacturer.

21.1 MAINTENANCE AND STORAGE OF THE pH SENSOR

After receiving the sensor, carefully remove all packaging, cut off the protective rubber layer from the sensor's upper membrane, if such is supplied. Wash the sensor with distilled water and wipe it dry with a clean paper towel. Afterwards, the sensor should be fastened on a stand and immersed in a 0.3 M KCl solution for several hours.



Warning!

Never let the pH sensor rest on its tip! Sensor's maintenance and storage should be done only while the sensor is fastened to a stand.

Inspect the lower part of the sensor for any gas bubbles. If any bubbles are present, eliminate them by shaking the sensor in a vertical motion. After the electrode is removed from its delivery package it should be stored on a stand and immersed in 0.3 M KCl solution, so that the upper membrane is submerged into the liquid.

If the sensor will not be used for a long period of time, it must be immersed in a 3 M KCl solution.



Warning!

Never store the electrode unclean!



Notice!

More detailed information about sensor use, maintenance and technical data can be found in a separate user manual from the sensor manufacturers.

21.2 MAINTENANCE AND STORAGE OF THE pO_2 SENSOR

After working with the sensor, its surface must be cleaned and washed with distilled water.



Warning!

Never store the electrode unclean!

Never let the pO_2 sensor rest on its tip! Sensor maintenance and storage should be done while the sensor is fastened to a stand.

While the sensor is not being used the membrane chamber must be filled with the O_2 electrolyte, and the electrode tip should be closed with the protective lid. Electrolyte must be changed every 3 months while storing the sensor. If the predicted period of storage will exceed 6 months, the sensor must be stored dry (i.e., without filling the membrane chamber with electrolyte). If the sensor is stored dry, we don't recommend connecting it to a power supply (i.e., pO_2 measuring device).

Sensor features

The sensors cable contact socket must be inspected prior to connection to make sure it is dry. Moisture, corrosion and dirt in the socket may result in false sensor measurements. Cable of the sensor also should be inspected, to ensure that there are no signs of insulation or socket damage.

Before commencing the calibration, visual inspection of the sensor membrane must be performed. If any dirt on the membranes surface is noticed it must be washed with distilled water.



Warning!

Never rub the pO₂ electrodes membrane with any materials (sponges, mops etc.) while washing it. This may result in membranes failure.

If any signs of damage are found on the membrane it must be replaced. The membrane should be replaced in case if the sensor reading inertness increases.

Do not use solutions containing alcohol for sensor cleaning. This may result in permanent damage of the sensor.



Notice!

More detailed information about sensor use, maintenance and technical data can be found in a separate user manual from the sensors' manufacturers.