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USER MANUAL

LABORATORY BIOREACTORS 5.1 & 5.2

User Manual
Ref: BioReactor5_rev 1.1

Please read carefully this manual before first use!

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WARNING: GENERAL INFORMATION AND SAFETY INSTRUCTIONS

It is necessary to strictly follow the instructions of use of this manual to ensure the proper functioning of the device or to exercise a possible resort to warranty.

To use this manual:

- Read these instructions carefully before using the device for the first time.
- Follow the instructions in the operating instructions.
- This manual is part of the product. Please conserve it.
- If you need to transfer this device, do not forget to attach the user manual.
- In case of loss, upon request, we will provide you with a new user manual.

Concerning these devices, some risks are to be taken into consideration (indicated by symbols):

This pictogram is intended to draw your attention to informations, observations of great importance, potential danger or a risk of personal injury.

This symbol informs the user of advice and additional information allowing him / her to make optimal use of the product.

1. Warranty

The manufacturer guarantees the correct operation of the provided system during the warranty period under the right conditions of transportation, storage, installation and operation, maintenance.

The warranty period shall be 24 months from the day of installation.

During the warranty period, the manufacturer repairs or replaces equipment and its parts free of charge. Shipping parts that are subject to warranty repair or replacement, is performed at the expense of the manufacturer.

Contact your supplier or Froilabo at +33 (0) 4 78 04 75 75 or e-mail us at commercial@froilabo.com

2. General Information

The following user manual enables safe and efficient handling of the further listed equipment. I t contains instructions, which describe the installation, operation and maintenance procedures of the BioReactor system and operation of the bioprocess controller. The user manual is an important part of the bioreactor equipment and should be kept accessible to the system operators at any time.

Warning!

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.

If hazardous or potentially hazardous products are used, only persons fully familiar with the equipment should handle these products. These persons must be capable to conduct an overall assessment of the potential risks.

In case if additional customer service or information is required, please contact your local supplier or Froilabo at +33 (0) 4 78 04 75 75 or e-mail us at commercial@froilabo.com

3. Description of the equipment

The 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, bottle holder;
- Bioprocess controller, equipped with a peristaltic pump system, power control unit, gas supply system, and a thermostat.

The bioprocess controller ensures:

1) Temperature (the temperature sensor (Pt100) is located in the bioreactor tank) regulation. The regulation is carried out by supplying a control signal either to the heating element or the electro-magnetic cooling water valve. Thermoregulation is carried out by circulation of thermostated water through the bioreactor's jacket, which is located in the bottom lid of the vessel;
2) pH control. Carried out by supplying base or acid solutions to the bioreactors using the bioprocess controller's peristaltic pumps;
3) DO (dissolved oxygen) control. Ensured by automatic adjustments of the stirrer's rotational speed, O2 enrichment of supplied gas and feeding rate adjustment. The actual DO value is monitored using a pO_2 sensor;
4) Foam control. Carried out by supplying an antifoam agent to the bioreactor's medium using the bioprocess controller's peristaltic pumps. The foam level is monitored using a conductivity sensor;
5) Feeding (of a substrate). Carried out using the bioprocess controller's peristaltic pump and the respective feeding rate/volume is controlled by the feeding profile, which can be set in the bioprocess controller;
6) Level control. Carried out using the bioprocess controller's peristaltic pump. The medium level is monitored using a conductivity sensor;
7) Mixing. Ensured by a magnetic drive (stirrer inner shaft and impellers contain magnets), the agitator is driven by a motor which is mounted on the top lid of the bioreactor.

4. Equipment installation and assembly

After receiving the equipment, we strongly recommend carrying out a visual inspection of the system. Inform the installation engineer or the manufacturer 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 bioprocess controller is between 5 ºC and 42 ºC;
3) Ensure a minimal space of between 15 - 20 cm at the back and on the sides of the bioprocess controller to eliminate any difficulties while 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, water drainage and exhaust gas lines are accessible.

4.1 INSTALLATION OF THE BIOREACTOR VESSEL

To ensure the correct functioning of the system, 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!

As was stated earlier the BioReactor system consists of the following two basic parts: (1) an equipped bioreactor vessel (2) and the bioprocess controller (see Figure 4.1).

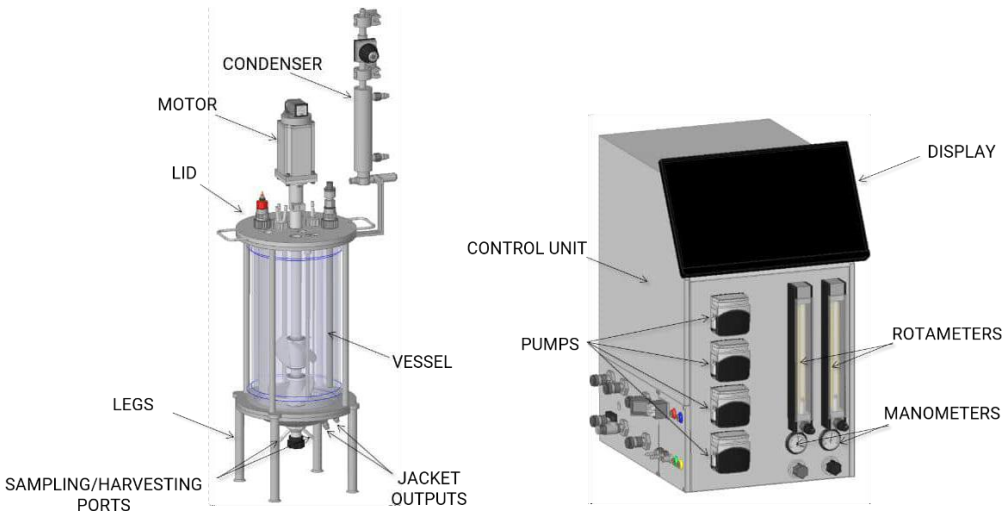

Figure 4.1. BioReactor system

While installing the BioReactor system, the vessel should be placed on the left side of the control unit as shown in Figure 4.1.

If it is necessary, and in order to make the bioreactors vessel more compact (so that it can be conveniently placed in an autoclave, etc.), the option of lowering the output air condenser has been provided (see Figure 4.2). To raise or lower the output air condenser, start by pressing the button indicated by the PUSH arrow 1 (see Figure 4.2 - A, B). Without releasing the button, lower (see Figure 4.2 - A, LOWER) or raise (see Figure 4.2 - B, RAISE) the output air condenser. Release the button only after achieving the desired position of the condenser.

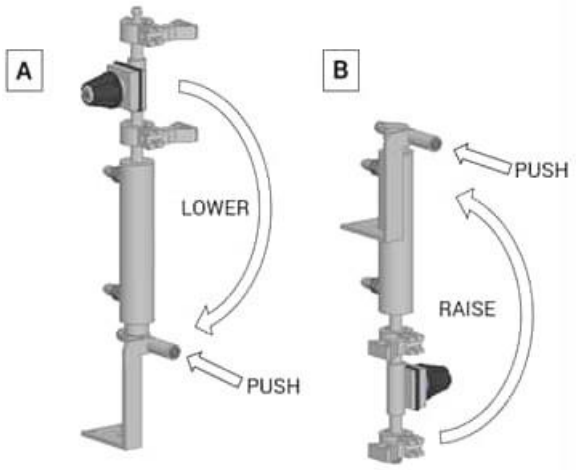

Figure 4.2. Raised (A) and lowered (B) output air condenser, with arrow indicators for raising or lowering it

After installing the bioreactors tank and the control unit, connect the respective inputs and sensors as depicted in Figure 4.8.

4.2 DESCRIPTION OF THE REACTOR LID AND PORTS

As a part of a standard set-up, the bioreactor's lid (see Figure 4.4) contains twelve ports for the following:

- Sensor connections (five ports);
- Liquid supply and septa port (*optional*) (three ports);
- Gas sparger and gas output port (two ports);
- Two spare ports that can be used according to the users preferences.

For liquid supply (see Figure 4.4), the user can use either one-way or three-way adapters (see Figure 4.3 - A and B, respectively). The standard set includes three adapters with a one-way inlet and one adapter with a three-way inlet.

Warning!

When tightening the lid ports, never use a spanner. All inlets and ports must be tightened by hand. If a efflux is detected, replace the respective adapter O-rings (gaskets). To ensure that no leaks occur greasing the O-rings with a vacuum lubricant (silicone grease) is advised.

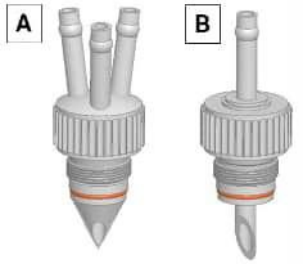

Figure 4.3. Ports with a one-way (A) and a three-way (B) inlet

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

Place the GASKET between the bioreactor's upper lid and the vessel in the cavity seen in Figure 4.4. The lid is placed on the reactor tank by holding it by its handles.

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!

When positioning the bioreactor, never hold it by the reactor inlets or by the equipment in the upper lids section. While positioning the vessel hold it by the bottom or by the lid handles.

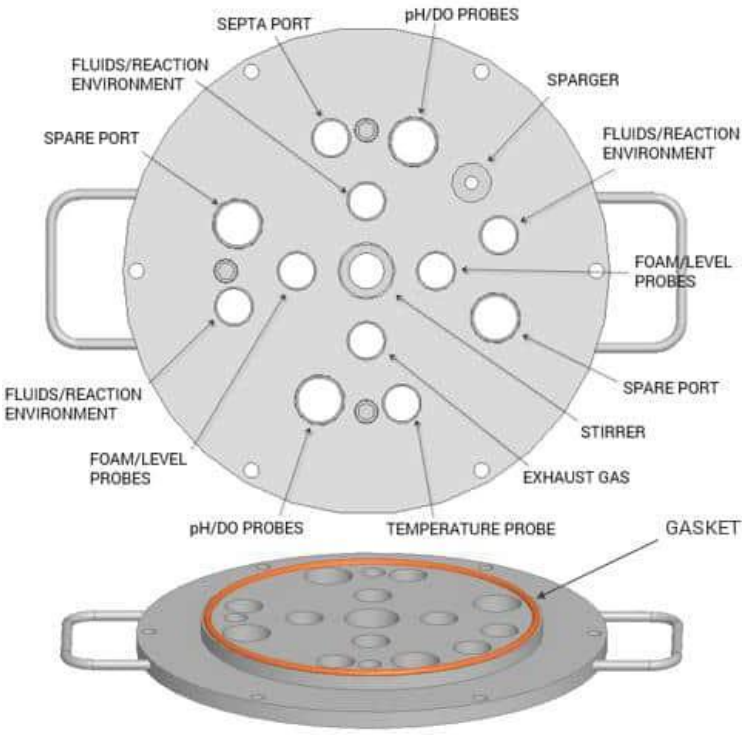

Figure 4.4. Chart showing the upper lid ports and gasket

While cleaning the bioreactors vessel, the occasional removal and cleaning of the stirrer impellers is recommended. In order to carry out this procedure, please follow the instructions described below (see Figure 4.5).

To remove the turbines, first loosen the screw on the upper lid that holds the sparger in place and then turn the bioreactors sparger from its initial position (see Figure 4.5 - A) to the position shown in Figure 4.5 - B. By pulling one turbine at a time in the direction that is opposite to the bioreactors lid, take off each turbine and silicon carbide bushing individually from the stirrers' axle (see Figure 4.6 - B).

The silicon carbide bushings and turbines should be placed back in the same order in which they were removed (Figure 4.6 – A, B). The order for placing the impellers back is of particular importance due to their different dimensions and paddle orientation (*if applicable*). The silicon carbide bushings may be swapped places without causing any problems.

Warning!

To avoid any damage or improper turbine work, ensure that no space has been left between the turbines and the bushings.

Always make sure, that the marine type impellers are mounted properly in respect to the direction of rotation (the top impeller should push the flow down, the bottom impeller should push the flow up).

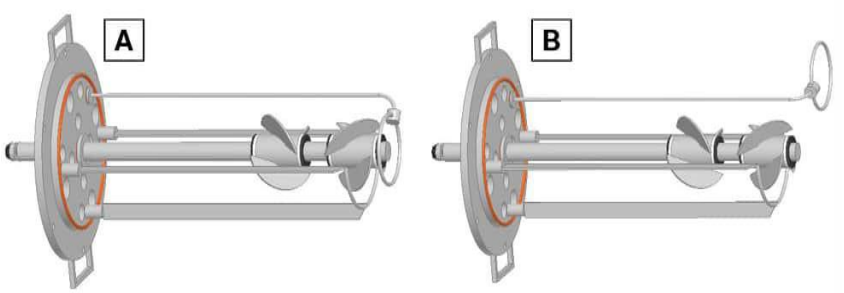

Figure 4.5. Two sparger positions. A - the operating position of the sparger; B - the sparger in its turned position thus allowing the removal of the turbines

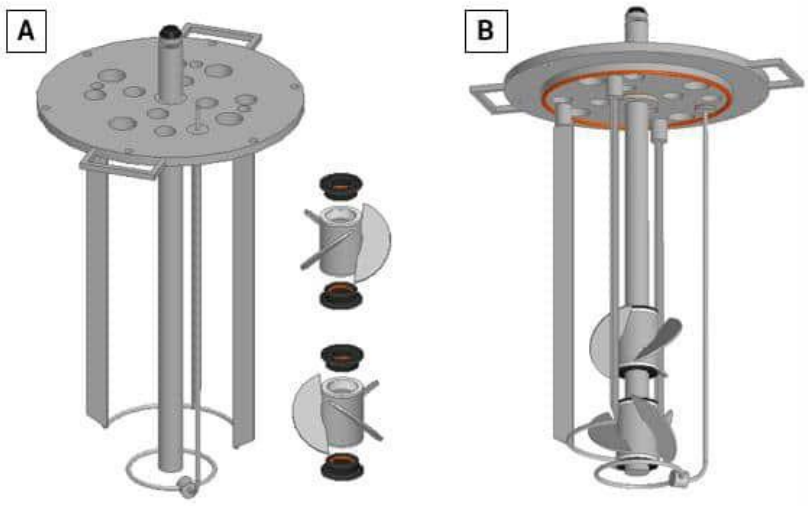

Figure 4.6. Removal of the reactor turbines. A - disassembled turbines and turbine bushings; B – correctly assembled stirrer paddle-type turbines if the turbines rotates counter-clockwise (looking from top of the lid) (for Rushton type turbines turbine rotation direction do not influence mixing quality!).

Correct positioning of the stirrer impellers is of utmost importance in order to ensure their proper work. The turbines should be placed opposite the stirrers inner axle magnets. The position is chosen according to the magnetic force principle. This procedure is carried out by moving the impeller along the bioreactors axle until the magnetic power fixes the rotor to the correct position. After the proper position of the impellers has been found, the silicon carbide bushings should be moved closely towards the turbines from its opposite ends. It is important to maintain the impeller at its correct position while the bushings are being pushed towards the rotors.

Warning!

To avoid any damage or improper operation of the stirrer, always make sure that the impellers and silicon carbide bushings are placed correctly and in line with the above-mentioned instructions.

Notice!

After assembling the stirrer it is recommended to perform a test to make sure that the stirrer is assembled correctly: when assembled, rotate the inner axle by hand, if this can be achieved without applying significant amount of force, then the stirrer is assembled correctly.

4.3 SENSOR INSTALLATION

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

The BioReactor system contains five sensors:

- Temperature - Pt100 temperature sensor;
- pH - Glass pH electrode;
- DO - Polarographic pO_2 electrode;
- Foam - Conductivity sensor;
- Level - Conductivity sensor.

The installation of pH and pO_2 sensors requires the use of port adapters as shown in Figure 4.7.

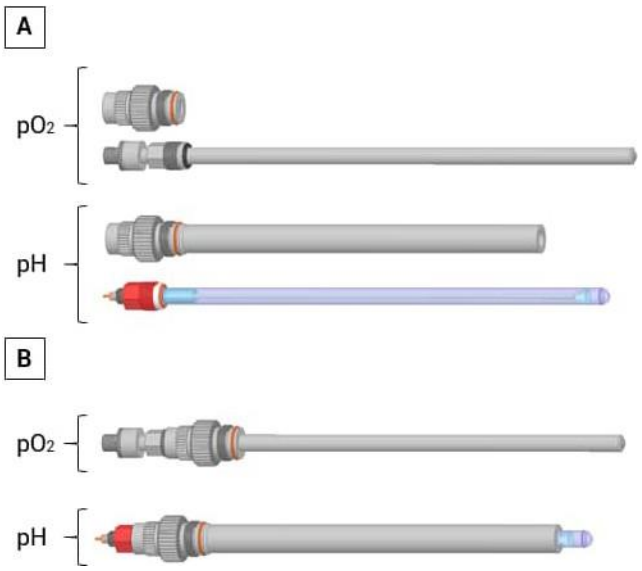

Figure 4.7. pH and pO_2 sensors with port adapters in a disassembled (A) and an assembled (B) state

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 manufacturers user manuals.

Warning!

Before inserting the sensors, ensure that the stirrer 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.

Always position the pH and DO sensors in ports, which are located further from the stirrer's axle.

Insert the pH and pO_2 sensors into the corresponding ports on the upper lid of the bioreactor and screw them in place until they are firmly attached.

4.4 SERVICE CONNECTIONS

The service connections for the BioReactor system can be found on the left side and in the rear part of the bioprocess controller (see Figure 4.8 and Figure 4.9). Use the supplied hoses and quick connections as shown in Table 4.1.

Table 4.1. Service connections

Service	Requirements	Connection type
Air	1.5 bar (Max 2.0 bar!!)	Quick connection
O_2	1.5 bar (Max 2.0 bar!!)	Compression connection
Water	1.0 - 1.5 bar (Max 2.0 bar!!)	Quick connection
Electrical power	208 - 230V, 50/60 Hz, one phase, 15 Amp (fluctuations do not exceed ± 10 %)	G series plug
Outlet air	Maximum overpressure 0.5 bar	Quick connection

Descriptions for the designations of Figure 4.8, Figure 4.9 and Figure 4.12 identify all of the service connections for the BioReactor system.

1) Using the supplied hose, connect the main water input (WATER) to the controller (Figure 4.8);
2) Using the supplied hose, connect the water output (DRAIN) to the bioprocess controller (Figure 4.8) and position it into the drainage system;
3) Using the supplied hoses, attach the connections TO JACKET / FROM JACKET to the bioprocess controller (Figure 4.8);
4) Using the supplied tubes and quick connections, attach the connections TO CONDENSER / FROM CONDENSER to the bioprocess controller (Figure 4.8);

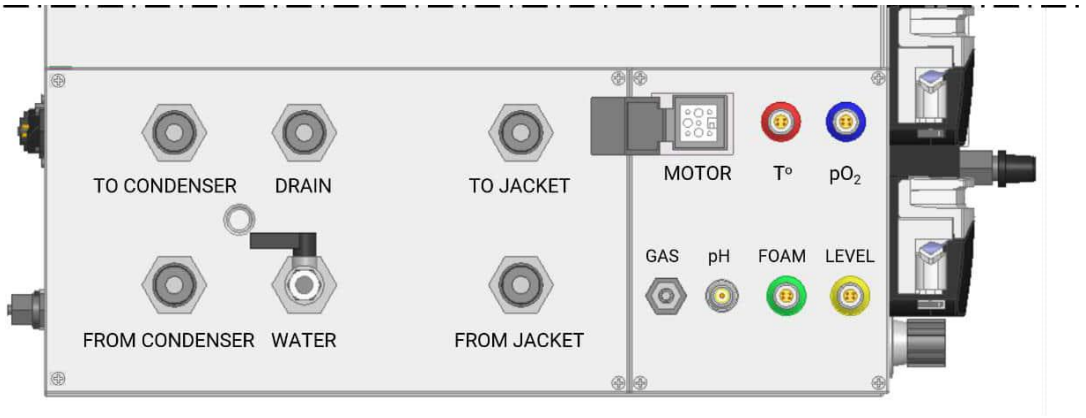

Figure 4.8. System water service connections (left side of the bioprocess controller)

Warning!

When connecting air and water supplies, in order to control the pressure, the installation of pressure reduction valves is recommended. For safety reasons do not exceed maximal limits of required flow pressures!

5) Using the polyurethane tube (SMC T0808), attach the AIR connection to the controller (see Figure 4.9);

6) Using the nylon tube (SMC T0806), attach the OXYGEN connection to the controller (see Figure 4.9).

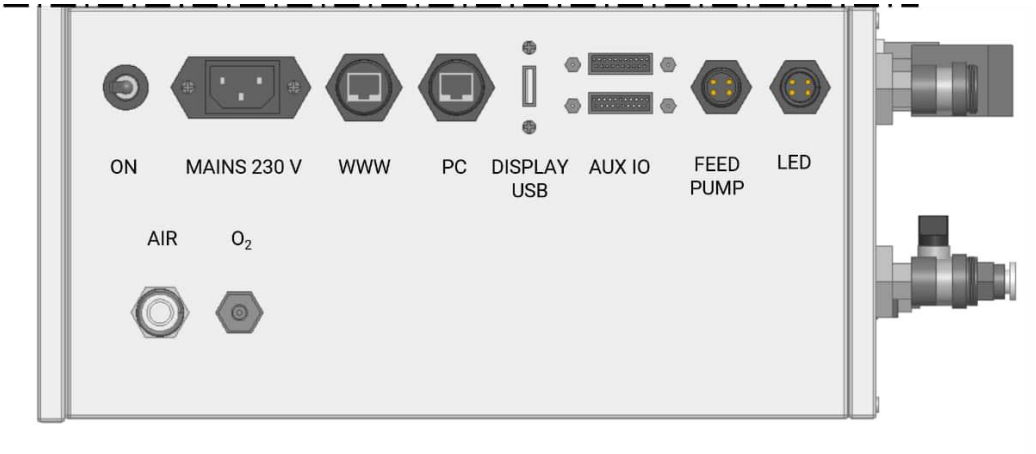

Figure 4.9. Air and oxygen service connections (back of the bioprocess controller)

4.5 VESSEL CONNECTIONS

After carrying out all of the activities listed in Section 4.4: "SERVICE CONNECTIONS", follow the instructions described below:

1) Attach the reactor inlet gas tube with an installed inlet air filter according to Figure 4.10 to the bioprocess controllers GAS outlet (see Figure 4.8). Connect the inlet air tube to the gas sparger port (see Figure 4.4);

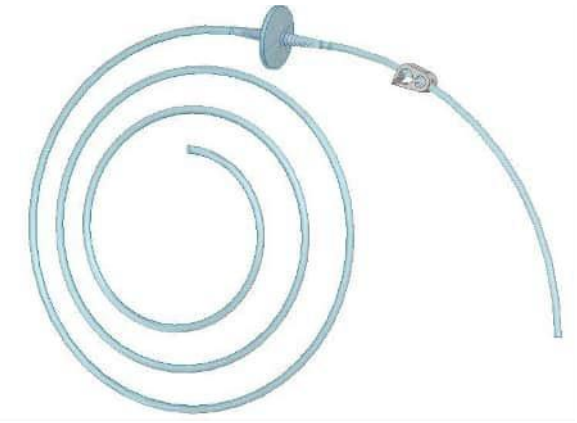

Figure 4.10. Inlet/outlet gas pipes and filter set

Notice!

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

2) Connect the outlet gas tube with its filter (see Figure 4.10) to the outlet air condenser at the location specified in Figure 4.11;

3) Connect the quick connections marked TO CONDENSER / FROM CONDENSER (Figure 4.8) to the outlet air condenser as shown in Figure 4.11;

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.

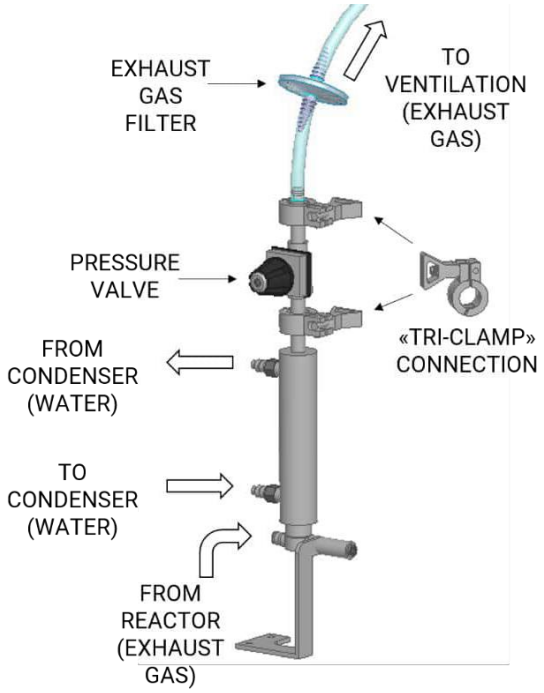

Figure 4.11. Outlet air condenser connections

4) Perform the connections TO JACKET/FROM JACKET (Figure 4.8) to the thermostating jacket outputs (see Figure 4.1). It is recommended to connect the TO JACKET tube to the lower thermostating jacket connection, but the FROM JACKET tube to the upper jacket connection;

5) Attach T, pH, DO, LEVEL and FOAM cables to the respective sensors. Then connect them to the bioprocess controllers respective ports shown in Figure 4.8;

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

1) Turn the motor's clutch into an appropriate position, so that the inner axles connection fits inside the motors connection (see Figure 4.12– A);

2) Place the motor on the stirrers axle (see Figure 4.12 – B);

3) To check whether the connections are fitted correctly, perform a visual inspection through the motors window (see Figure 4.12 – C);

4) Using minimal force carefully tighten the motor screw (see Figure 4.12 – D).

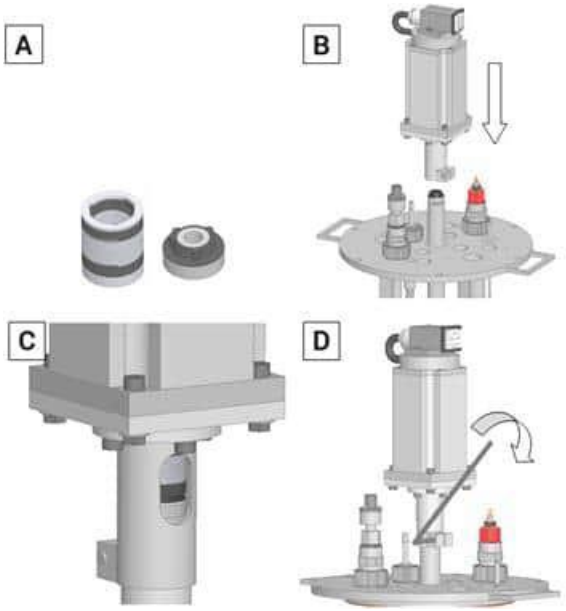

Figure 4.12. Motor installation

To install the peristaltic pump tubing, see Section 11.1: "TUBING".

4.6 POWER CONNECTION

In Section 4.5: "VESSEL CONNECTIONS" electrical power connections for the motor and system sensors were described. In order to start working with the BioReactor system, make the following connections (see Figure 4.9).

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 network switch is in OFF position.

Make sure that the equipment is grounded.

Perform the service and device connections on the backside of the bioprocess controller:

1) Connect the electrical cable to the POWER 230 V input;
2) Connect the internet cable (if required) to the WWW input;
3) Connect the computer cable (if required) to the PC input;
4) Connect the external feeding pump (if required) to the FEED PUMP input.

It should be noted that the standard set of the bioprocess controller, includes a built-in internal UPS (uninterruptible power source). In case if any interruptions in the power supply occur, the UPS can ensure the operation of the controller for up to 15 minutes. Online data storage in the database is ensured within this time interval.

5. Specifications

In Table 5.1 the main specifications of the BioReactor system are listed.

Table 5.1. Main specifications of the BioReactor system

Net weight	Control unit	30 kg
	Vessel	15 kg
Total dimensions		Width 0.7, depth 0.7, height 0.7 (m)
Controller		15" colour touchscreen
		Flexible software for process control
		Built-in Wi-Fi router for connection with SCADA and other computer software for wireless control
		Built-in UPS power supply source
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	During the process, the temperature in the bioreactor is controlled trough the jacketed bottom surface. Jacketed bottom surface temperature are controlled with by the thermostated water. The control elements are the electric heater and the electric magnetic cooling valve. All thermoregulation elements are placed in a separate unit, which is connected to the water pipe, sewerage and jacket, which covers the bottom of the bioreactor
	Type of control	PID control
	Range	In normal mode the water supply at a temperature of between 16 – 52 °C is used as cooling water (with an insulation jacket of up to 70 °C).
Agitation	Drive	105 W 24V BLDC motor with electronic control
	Type of control	PID control or manual settings
	Range	40 – 1000 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 – 8.6 h L/min
Outlet air	Air filter	0.22 µm filter sterilized by autoclaving
Mixing two gases	Type of control	Manual and/or automated with electric magnetic valves
Peristaltic pumps		Four 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
Level	Sensor	Conductivity sensor
	Control performance element	Peristaltic pump
pH (facultative)	Sensor	Sterilizable gel pH sensor
	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, inlet air enrichment with oxygen and feeding speed
	Type of control	PID control
	Range	0 - 150 % from the air content

6. Work with the bioprocess controller

The bioprocess controller ensures control and supervision of the BioReactor system.

The control unit ensures that specific parameter set-points (SP) (T, pH, DO etc.) can be controlled and their actual process values (PV) can be visualised. The operation activity and actual values of some control actuators (Valves, Pumps, etc.) is possible. Extended (*optional*) version of SCADA ensures remote control of most bioreactor's control parameters and actuators.

6.1 STARTING THE WORK

To start working with the bioprocess controller follow the instructions listed below:

1) Turn on the controller by moving the ON/OFF switch at the back of the control unit (see Figure 4.9). The Company logo will appear on the main screen of the bioprocess controller. Touch the screen to proceed;
2) The REACTOR window will appear, see Figure 6.1. More detailed information about the functionality of this window is given in Section 6.2: "REACTOR WINDOW";

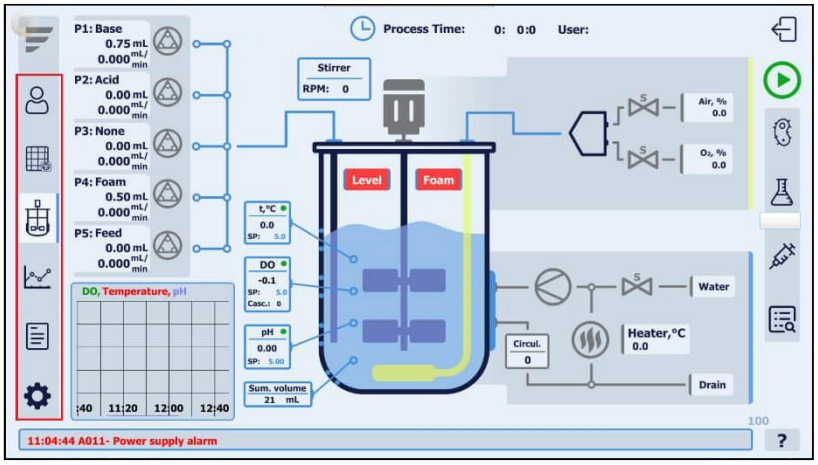

Figure 6.1. REACTOR window

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) The MAIN MENUof the bioprocess controller is located on the left-hand side of the user interface (highlighted in Figure 6.1). Press the ADMINISTRATION button, thus opening the ADMINISTRATION window, where the use can log in into the system, see Figure 6.2;

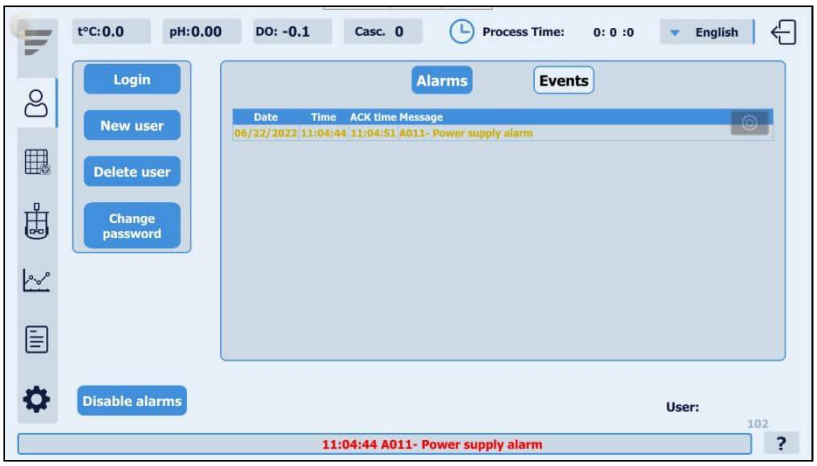

Figure 6.2. Control unit's ADMINISTRATION window

4) Choose the desired user entry (by default Viewer, Operator or Administrator) (Figure 6.3) and enter the respective password after clicking on the blank space next to the word password (Figure 6.4), press ENTER and OK.

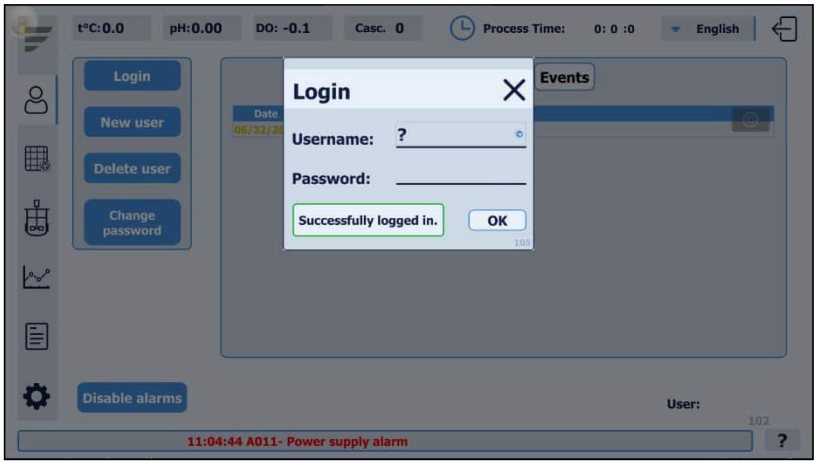

Figure 6.3. Access level window

By default, the password for the above mentioned access control levels are pre-set as follows:

User	Password
Operator	1111
Controller	2222
Admin	1234

Notice!

The virtual keyboard allows entering desired values by pressing the respective characters and digits. Pressing ENTER confirms and saves the entered value.

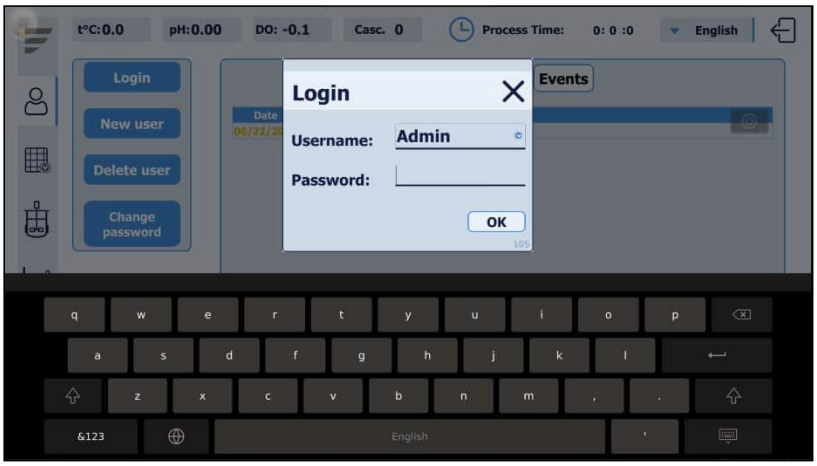

Figure 6.4. The virtual keyboard

The Administrator access level allows to:

- Create new users.

After pressing the NEW USER button, a pop-up window will appear in which it is possible to enter the new user's name, password and access level (Privilege – Admin, Controller, Operator). Only Admin privilege users can create New User, Delete users and Change passwords. Controller privilege users can operate with every bioprocess controller operation element except the mentioned above. the Operator privilege users can access only the REACTOR, SETTINGS (1ST and 2nd WINDOW), FEEDING, RECIPE and TREND window.

To confirm this action - press OK, see Figure 5.6.

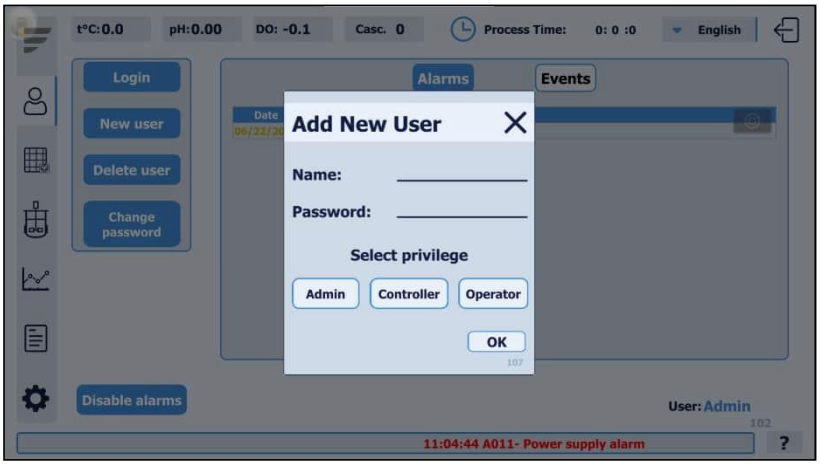

Figure 6.5. New user window

- Delete user.

After pressing the DELETE USER button, a pop-up window will appear in which it is possible to select the desired username, which needs to be deleted. By pressing the DELETE button confirm the action, see Figure 6.6.

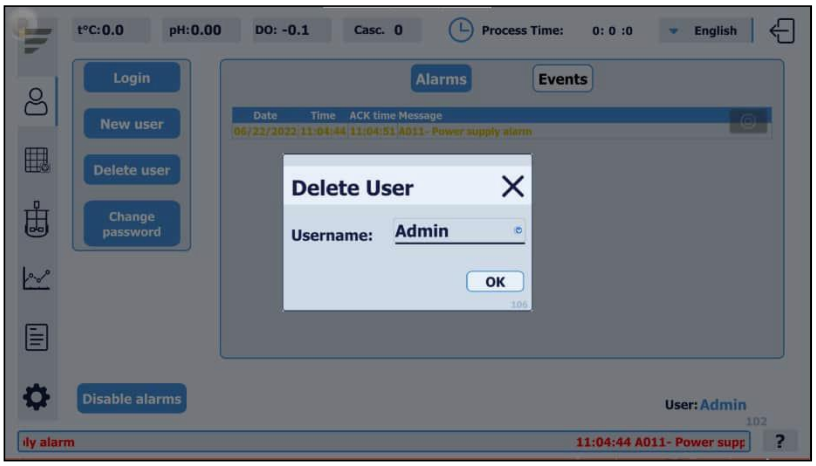

Figure 6.6. Delete user window

- Change password.

After pressing the CHANGE PASSWORD button, a pop-up window will appear in which it is possible to select the desired Username, whose password ought to be changed. Enter the new password and press OK to confirm the action, see Figure 6.7.

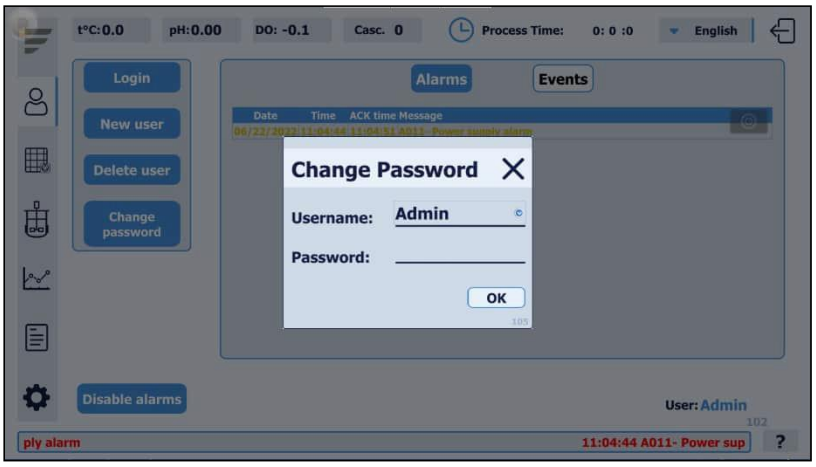

Figure 6.7. Change password window

To log out of the system the user can press on the LOG OUT button, which is seen in the top right corner of the software interface (highlighted on Figure 6.8).

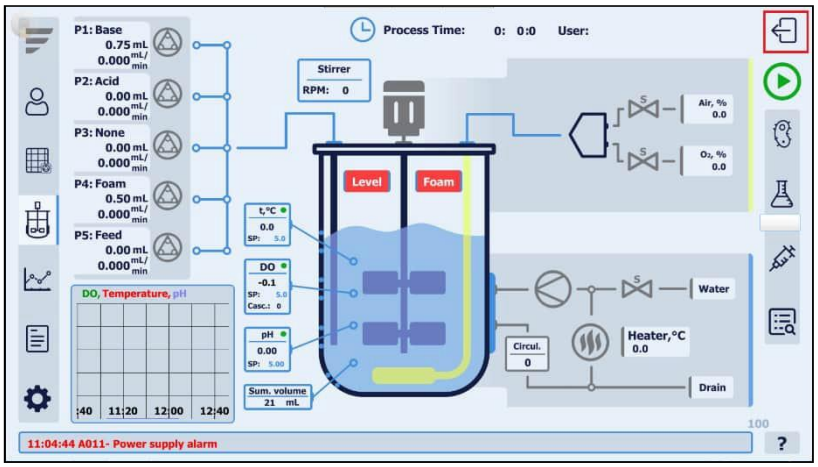

Figure 6.8. REACTOR window (log out button highlighted)

6.2 REACTOR WINDOW

By using the access path MAIN MENU → REACTOR open the REACTOR window (see Figure 6.9).

In the REACTOR window all of the most important process parameters can be easily configured and monitored.

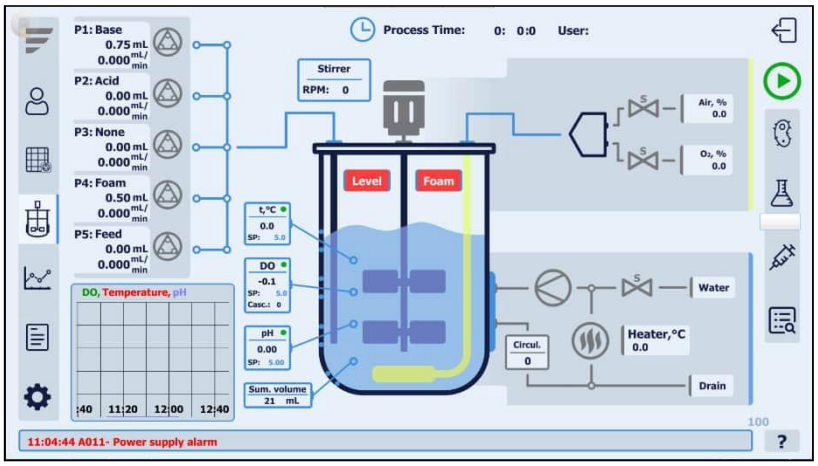

Figure 6.9. REACTOR window

7. Temperature control

Process temperature control is carried out by supplying a control signal to either the heating element (700 W) or the cooling water electro-magnetic valve. For temperature regulation the respective sensor (Pt100) has to be installed in the bioreactor tank. The heating element and the electro-magnetic cooling water valve are located in the bioprocess controller's thermostat. The circulation pump (also located in the thermostat) maintains the water flow through the bioreactors jacket, which is installed in the bioreactor bottom lid.

Notice!

Temperature sensors (Pt 100) are pre-calibrated prior to delivery, and repeating of the calibration procedure in unnecessary.

7.1 BIOREACTOR TEMPERATURE CONTROL

The use can set-up the set-point for temperature in the REACTOR window within the temperature control window, highlighted in Figure 7.1.

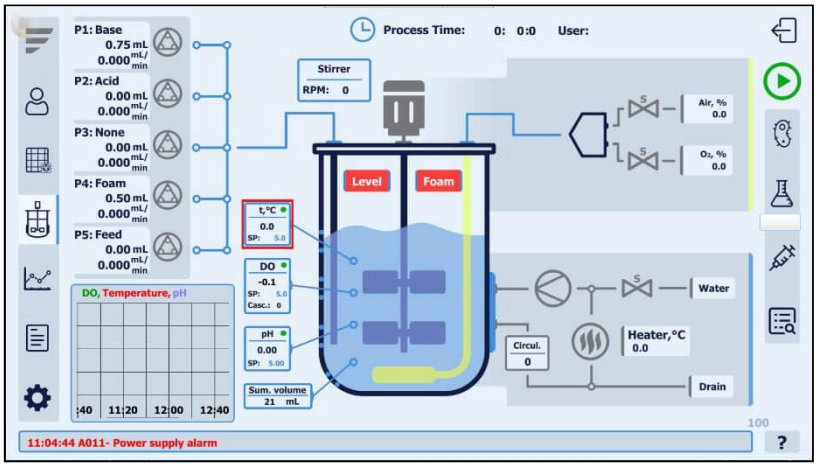

Figure 7.1. Temperature configuration window

Adjust the temperature set-points by clicking on the blue digits next to the abbreviation SP and enter the necessary values, confirm your action by pressing ENTER.

Alternatively, the temperature set-point can be configured in the SETTINGS window. Using the access path MAIN MENU → SETTINGS (1st WINDOW) → SETPOINTS. Adjust the set-points by clicking on the respective digits (highlighted in Figure 7.2).

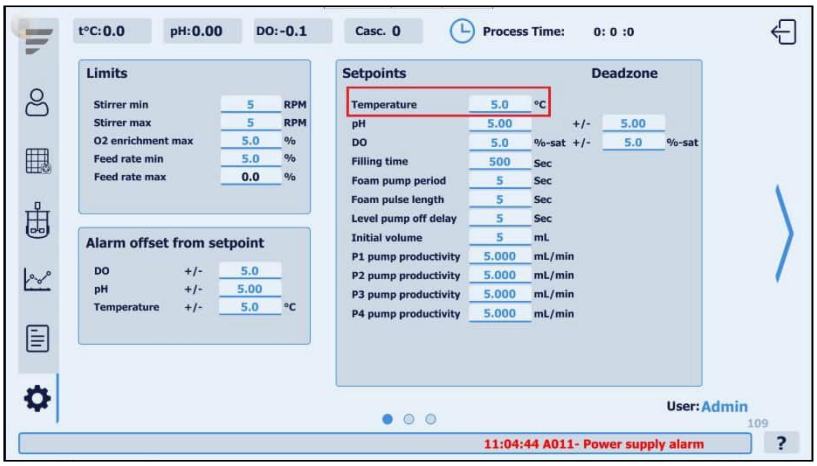

Figure 7.2. SETTINGS window

Temperature control can be carried out in either Manual or Automatic mode. Note that the water connections and temperature sensor wiring (see Sections 4.4: "SERVICE CONNECTIONS" and 4.5: "VESSEL CONNECTIONS") should be performed prior temperature monitoring and control. Liquid in the reactor should be agitated (see Section 8: "Agitation control") to maintain consistent temperature distribution. Using the access path MAIN MENU → REACTOR, click on the HEATER or WATER valve symbol (highlighted in Figure 7.3) and select the desired control mode - Manual or Automatic in the pop-up window.

If an element is switched to manual mode a letter M will appear above it in the REACTOR window, highlighted in Figure 7.3. Green colour indicates the currently active operation elements, see Figure 7.3.

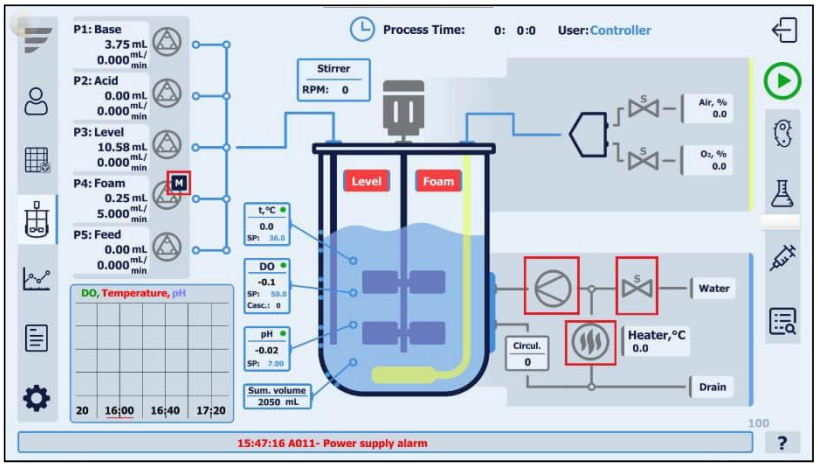

Figure 7.3. Symbols of the circulation pump, water valve and heater

While in Manual mode, the cooling or heating actions can be implemented by switching ON the respective control element (water valve, heater and circulation pump).

While in Automatic mode, automatic temperature control at pre-set set-points is implemented whilst the fermentation process is enabled. The respective control elements operate according to the PID algorithms and individual PID coefficient values (see Section 19.1: "SETTING UP PID PARAMETERS").

Warning!

Temperature control can be implemented only if liquid flow is present in the thermostat loop. The circulation sensor measures the mentioned flow rate. If the heater is turned on, but no flow is present at-least during 30 seconds, the system will automatically terminate further temperature control. Refer to the TROUBLESHOOTING section for more advise.

8. Agitation control

The BioReactor systems agitation specifications:

- Stirrer rotational speed interval: 40 - 1000 rpm;
- Drive type: magnetic;
- Impeller type: standard Rushton.

The stirrers 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 cannot be equal to each other and the Min parameter has to be smaller than the Max parameter. The stirrer rotational speed can be regulated within the range defined by the Min and Max parameters (to set them up use the following access path: MENU → SETTINGS (1ST WINDOW) → LIMITS highlighted in Figure 8.2).

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

8.1 CONTROL SETTINGS

The stirrer control window can be entered by using the following access path: MAIN MENU → REACTOR 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 8.1. Also, the desired operation mode – Manual/Automatic can be chosen.

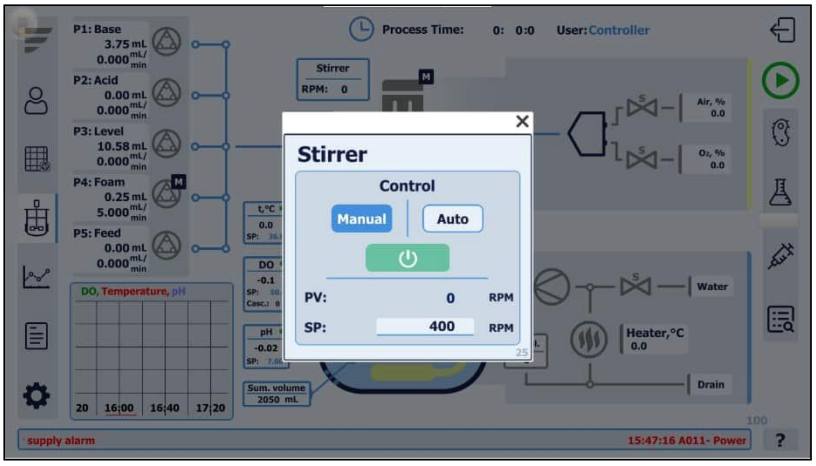

Figure 8.1. Stirrer's control window

The stirrer speed setting adjustment for DO control can be performed using the path: MAIN MENU → SETTINGS (2ND WINDOW) → DO CASCADE. More detailed information about cascade control can be found in Section 8.2: "DISSOLVED OXYGEN (DO) CONTROL BY STIRRER ROTATION RATE" and Section 13: "Dissolved oxygen (DO) control". To set-up the Min/Max parameters for stirrer rotational speed control, use the following access path: MAIN MENU → SETTINGS (1ST WINDOW) → LIMITS → STIRRER (highlighted in Figure 8.2).

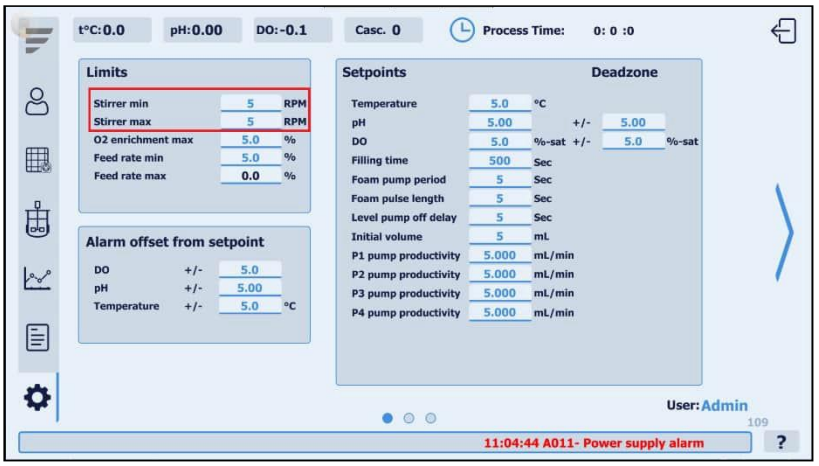

Figure 8.2. Stirrer rotational speed Min/Max parameter setting window

8.2 DISSOLVED OXYGEN (DO) CONTROL BY STIRRERROTATION 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 pO_2 value are defined, the stirrer operates in such a way, to maintain the process pO_2 value at: SP ± DZ. If the pO_2 value is within the aforementioned range, the stirrer's rotational speed remains constant. If the pO_2 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 13: "Dissolved oxygen (DO) control".

9. Air/O_2 supply settings

The AIR and O_2 gas flow and pressure control equipment is located on the front panel of the bioprocess controller and consists of:

- Gas flow indicators/regulators (rotameters);
- Manometers;
- Pressure regulators (see Figure 9.1).

Rotameters ensure the manual regulation of the respective gas flow rate. Information about rotameter scale calibration (relationship to flow rate in liters per minute) can be found in the technical documentation that is supplied alongside with the equipment.

Pressure regulators ensure the regulation of the respective gas pressure before entering the rotameter (manometers serve as pressure indicators). Pressure regulators also allow manual regulation of the respective gas flow. Normally, gas flow rate regulation is carried out by rotameters, but in some cases using pressure regulators, it is possible to adjust the pressure of the inlet gas (to 0.05 MPa or higher), in result increasing or decreasing the gases flow rate.

Manometers enable visual monitoring of the respective gases pressure (in MPa) before entering the rotameter.

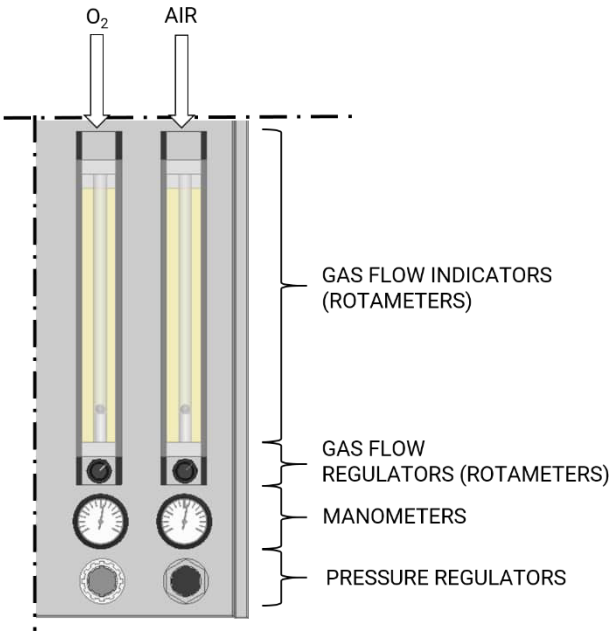

Figure 9.1. Gas flow control equipment

To set-up the air/O_2 supply, follow the instructions listed further:

1) Access the REACTOR window and locate the AIR and O_2 sections in the top-right corner of the window. By pressing the respective gas valve symbol, the control window for the respective valve will appear, see Figure 9.2;

2) After switching on the Manual control of the respective valve, enable the gas flow by turning ON the valve;

3) Based on the respective manometer reading, set-up the desired gas pressure using the pressure regulator (see Figure 9.1);

4) Set-up the desired gas flow rate using the respective rotameter (taking into account the calibration and correction values based on pressure, temperature and density of the applied gas).

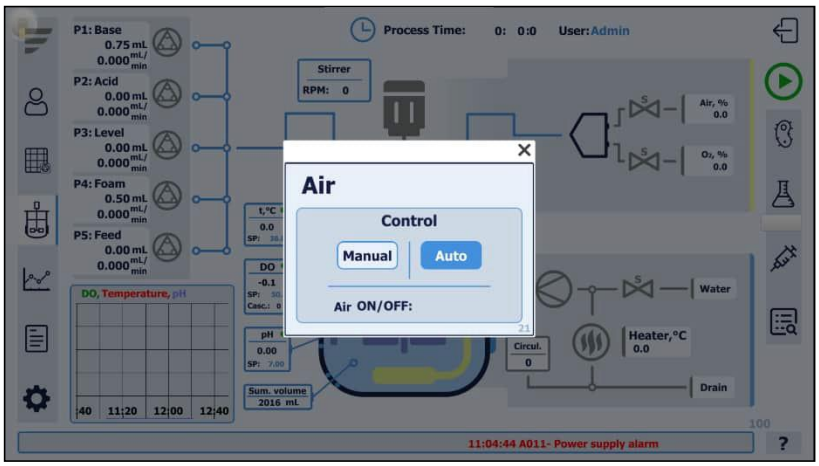

Figure 9.2. Gas control window

10. Two gas supply control

The BioReactor system provides possibility of mixing two gases (air/O_2) to obtain a desired O_2 concentration in the inlet gas flow. This can be implemented in two ways: (1) manual two gas mixture supply and (2) two gas mixture supply via automated control of DO, see Section 13: "Dissolved oxygen (DO) control".

The principle of setting-up mixing of two gasses is similar to that described in Section 9: "Air/O_2 supply settings" (in the case of (1) manual two gas mixture supply), and in Sections 13: "Dissolved oxygen (DO) control and 16: "Dissolved oxygen cascade control", (in the case of (2) two gas mixture supply via automated control of DO (pO_2)). In such cases only the types and combinations of the respective gasses will differ. To exercise such an option, please see the respective sections (Sections 9: "Air/O_2 supply settings", 13: "Dissolved oxygen (DO) control" and 16: "Dissolved oxygen cascade control").

10.1 DISSOLVED OXYGEN CONTROL BY MIXING OF AIR/O_2

The automated Air/O_2 mixtures control is performed by adjusting the respective gas valves open time. The control is implemented in such manner, so that O_2 concentrations range of 0 – 100 % can be achieved while the summary flow remains constant.

When the O_2 concentration in the inlet flow needs to be increased, the open time of the O_2 gas valve is increased, while the Air gas valves open time is decreased proportionally. This results in O_2 concentration growth while the summary gas flow remains constant. If the DO PV value remains > than the DO SP value and the O_2 gas valves open time reaches its Max limit, then the DO control will be implemented further by next cascade (if the next cascade was chosen). If the DO PV value remains < than the DO SP value and the O_2 gas valves open time reaches its Min limit, then the pO_2 control will be implemented by the previous cascade (if the next cascade was chosen).

To set-up the Max parameter for oxygen enrichment control, use the following access path: MAIN MENU → SETTINGS (1st WINDOW) → LIMITS → O_2 enrichment max (highlighted in Figure 10.1).

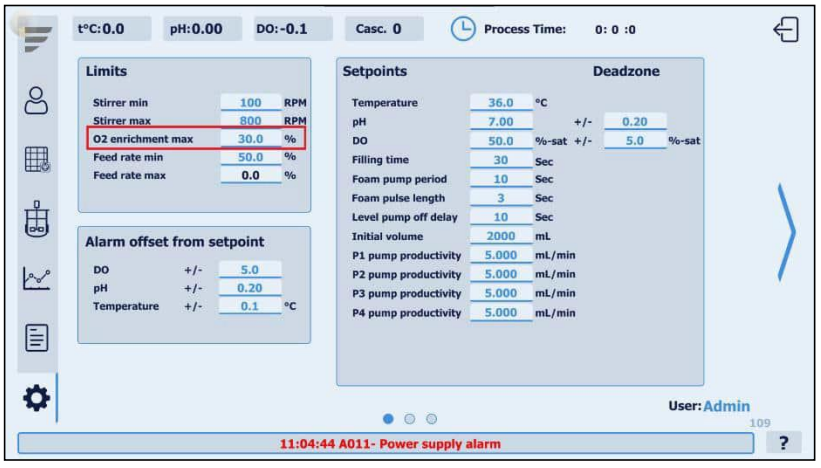

Figure 10.1. Settings for O_2 enrichment

11. Pump control

By default, the BioReactor system includes four peristaltic pumps for pH, foam, medium level regulation and substrate addition (feeding).

11.1 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. Their exterior diameter and wall thickness should not exceed 6-8 mm and 1.5 mm, respectively;
2) Open the peristaltic pump's head by lifting up the lid. Place the silicone tube around the rotating part of the pumps head and place both ends into their outlets;

Notice! Bioreactor's peristaltic pumps operate clockwise in default.

3) Close the pump head with an installed silicone tube by lightly pressing the lid down until you hear a click;
4) Connect one end of the silicone tube to the titrant bottle and connect the other end to the desired bioreactor port.

11.2 PUMP PRODUCTIVITY

The productivity of each peristaltic pump is determined during the calibration process (see Section 11.4: "PUMP CALIBRATION").

Notice!

Choose appropriate operation speed for every pump. If a pump is intended to operate with a very low productivity, we recommend at first testing whether or not the pump will be capable of rotating at a desired speed. Also, we recommend performing this test while the tube is mounted on the peristaltic pump's head. This is advisable, because different tube material and diameter can cause different resistance, which may restrict the pump from rotating.

Higher levels of productivity can be achieved by using tubes with a larger diameter.

To set-up the productivity of each peristaltic pump (excluding the feeding pump), use the following access path: MAIN MENU → SETTINGS (1st WINDOW) → SETPOINTS → P1-P4 pump productivity (highlighted in Figure 11.1).

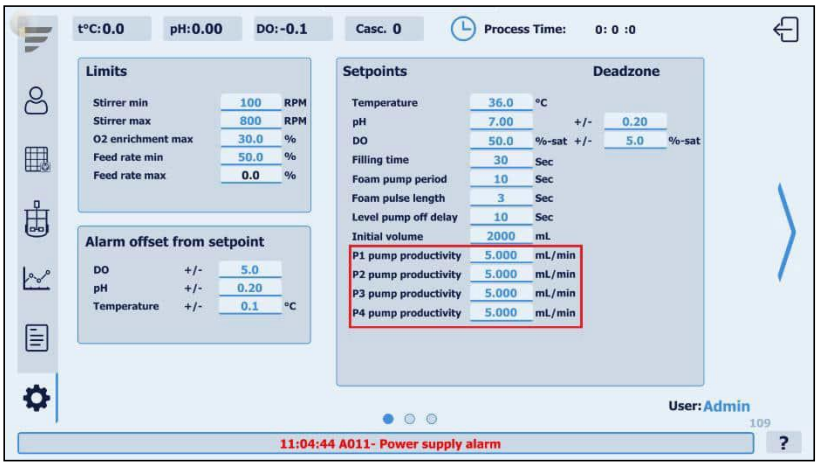

Figure 11.1. Peristaltic pump productivity settings

The pump productivity settings correspond to each individual pumps liquid transfer rate, which will be maintained in case if the pump will be enabled by the system during pH, Level and Foam regulation.

11.3 PUMP CONFIGURATION

To access the pump configuration menu, use the following access path: MENU → SETTINGS (2nd WINDOW) → PUMP CONFIGURATION, see Figure 11.2.

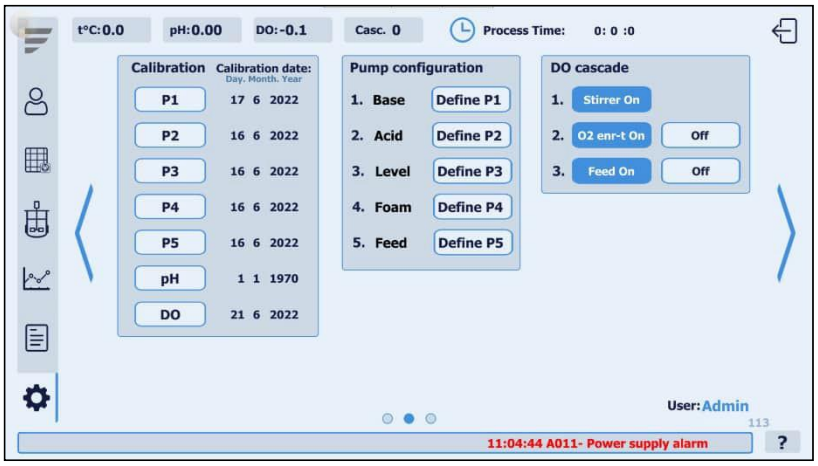

Figure 11.2. PUMPS and pO_2 CASCADE control window

In the pump configuration menu it is possible to define, what function each individual pump will carry. To configure a pump press the respective button (Define P1, P2, P3, P4 or P5), then select the desired function Base, Acid, Feed, etc. Keep in mind that you can choose only one pump for a specific function.

11.4 PUMP CALIBRATION

Follow the instructions listed below before performing pump calibration:

1) Mount the tube, which is intended to be used on the desired peristaltic pump head and attach the reagent bottle to the suction end of the tube;

Notice!

When inserting the tubes into the pump head, please remember that the peristaltic pumps are operating clockwise.

2) Fill the tube with a solution that is intended to be used for pump calibration, e.g. water. 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 bioprocess controllers REACTOR window. Set the pump, which is intended for calibration (base, acid, antifoam, level or feed (P1-P5)) to automatic mode (click on the respective pump symbol and press AUTO);

4) Open the calibration window by using the access path MENU → SETTINGS (2nd WINDOW) → CALIBRATION ;

5) Use buttons P1, P2, P3, P4, and P5 to select the desired pump for calibration (see Figure 11.3). The P5 pump is the external pump* (controlled by an analogue signal), pumps P1 – P4 are built into the bioprocess controller;

6) In the calibration window define the desired amount of calibration points and the respective speeds and calibration time for each point (see Figure 11.3);

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.

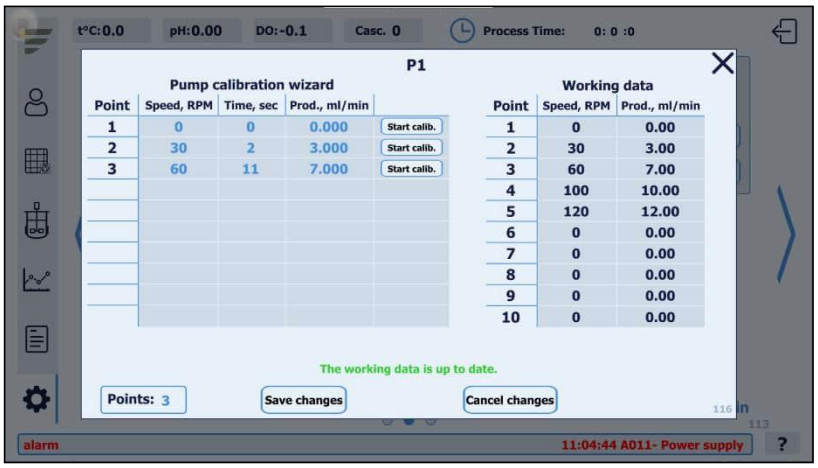

Figure 11.3. Pump calibration window

7) Pump calibration point amount selection depends on the pre-set function of the pump – in case if the pump is used for maintaining pH, Foam and Level, it is recommended to choose at-least three calibration points. For feeding at-least seven calibration points are recommended;

8) By pressing the START CALIBR. button the calibration procedure enables. During a pre-defined amount of time the pump operates using the defined settings (see Figure 11.4);

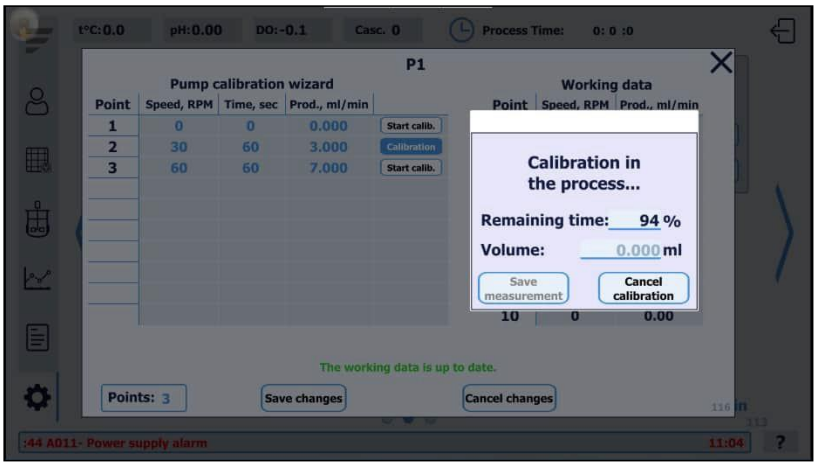

Figure 11.4. Pump calibration procedure

9) Remaining Time: shows the remaining time of the calibration cycle;

10) After calibration is complete, enter the pumped volume in *ml* into the respective section of the calibration window (Volume).

11) By pressing the Save measurement button the user can save the calibration data (see Figure 11.5). By pressing the Cancel calibration button the user can discard the newly entered calibration values.

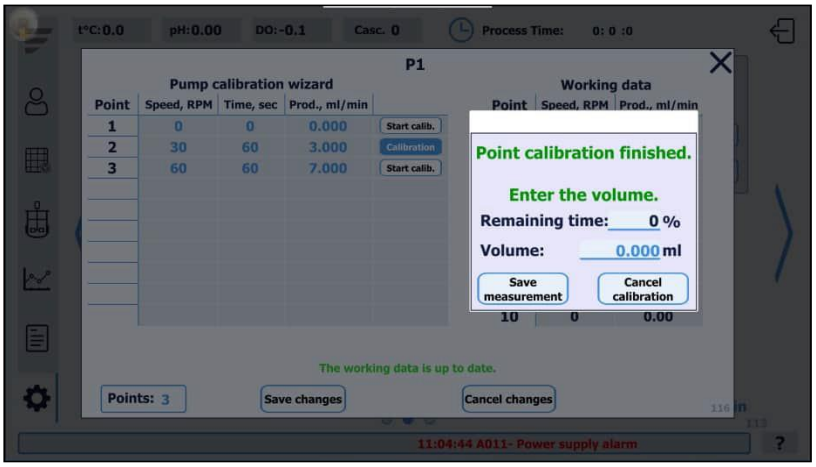

Figure 11.5. Pump calibration procedure finished

12) After the calibration of all envisioned points has been carried out, the user has to save the calibration data to the system by pressing the Save Changes button. Alternatively, all changes and calibration data can be discarded by pressing the Cancel Changes button (see Figure 11.6).

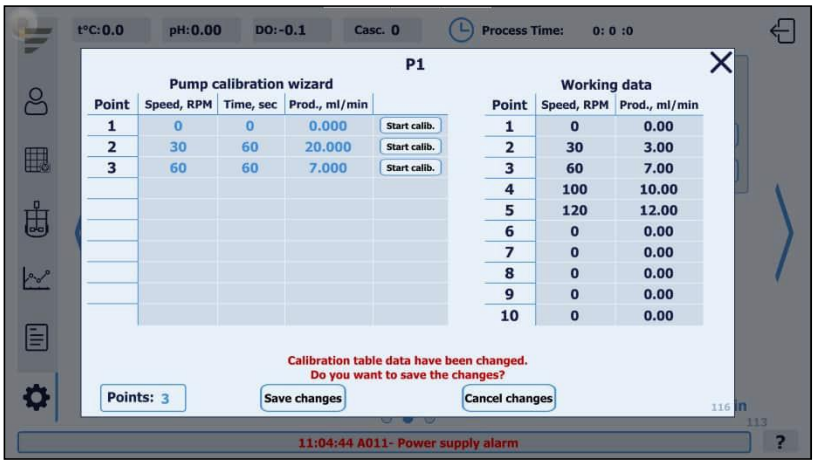

Figure 11.6. Saving pump calibration data

13) To finish the calibration procedure close the calibration wizard's window.

11.5 PUMP CONTROL FROMTHE BIOPROCESS CONTROLLER

Pump control from the bioprocess controller can be implemented in two ways: manual (Manual) or automatic (Auto). Open the REACTOR window and navigate to the required pump control window by clicking on the respective symbol (see Figure 11.7). By selecting Manual the option of enabling the pump in manual mode is provided. If the Auto mode is selected, the pump manual controls are suppressed and it will operate only during the fermentation process, in accordance with the respective control settings.

Additionally, the user can reset the pumped volume value within the pump control window by pressing the reset button.

The pump can be automatically enabled to operate with the maximal rotation rate by pressing the fast forward button, within the pump control window.

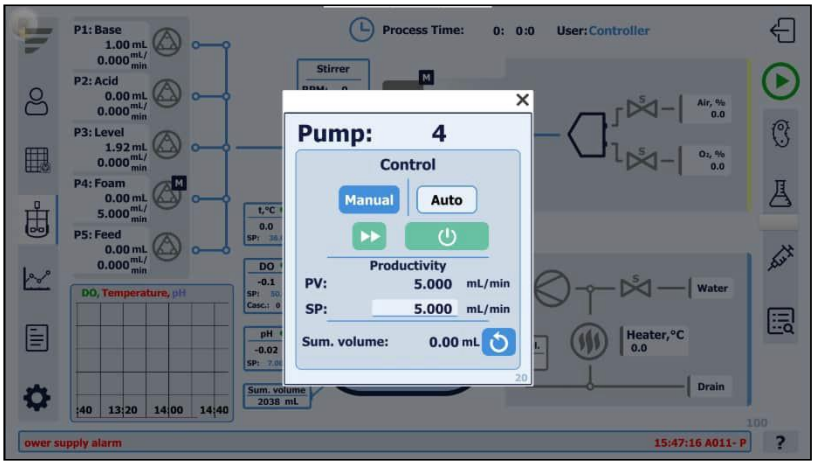

Figure 11.7. Pump control window

12. 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 bioprocess controller's peristaltic pumps (see Section :11 "Pump control").

Notice!

Operation and maintenance of the respective sensors has to be done according to the guidelines described in Section 29.1: "MAINTENANCE AND STORAGE OF THE pH SENSOR".

12.1 PROCESS PH CONTROL

To access the pH control window, open the REACTOR window, locate the pH section (highlighted in Figure 12.1) where the SP value can be set.

Or use the following access path: MENU → SETTINGS (1st WINDOW) → SETPOINTS to adjust the parameter set-point (see Figure 12.2).

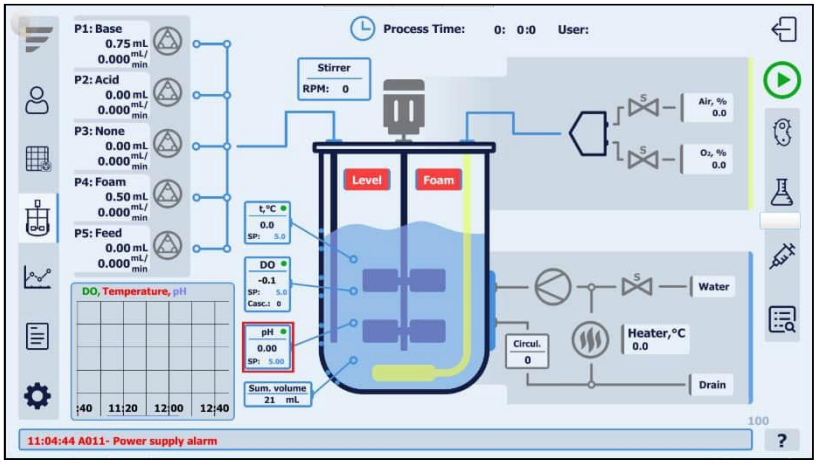

Figure 12.1. pH value indication within the REACTOR window

Detailed information on process control settings, including pH control, can be found in Section 23.1: "CONTROLLER SETTINGS FOR STARTING THE PROCESS".

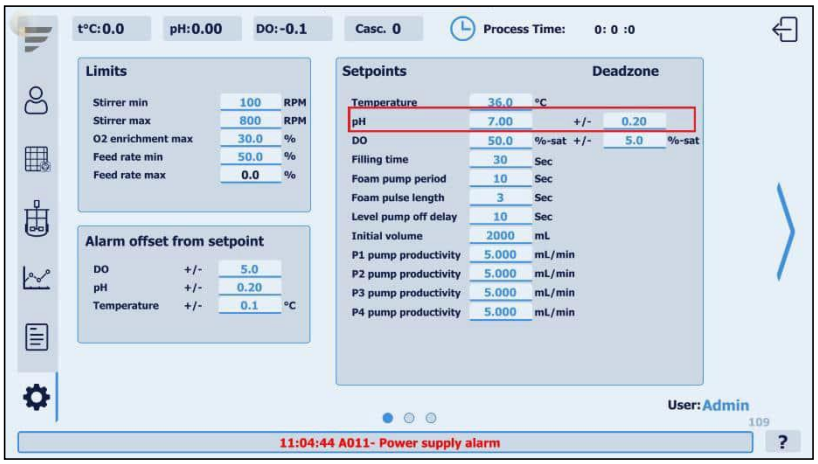

Figure 12.2. Window for setting the process pH value

12.2 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 and whipped dry with a paper towel before submerging it into the buffer solution or before storage.

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.

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

1) By using the access path MENU → SETTINGS (2nd WINDOW) → CALIBRATION → pH, open the pH calibration wizard (see Figure 12.3);

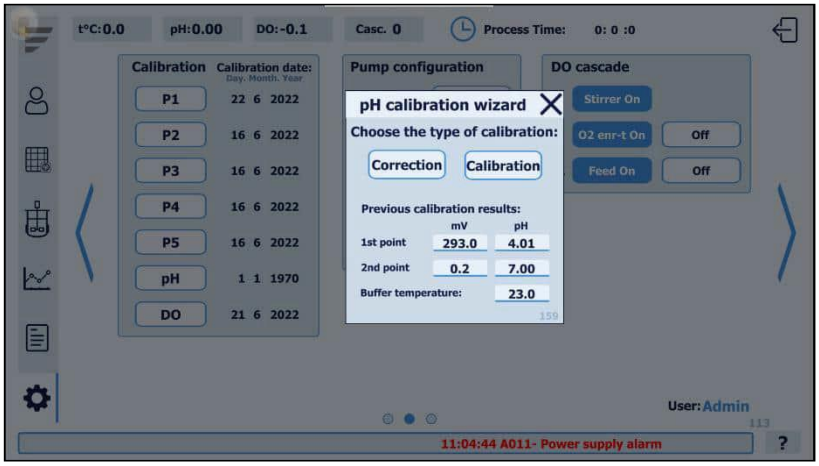

Figure 12.3. pH Calibration wizard

2) Two options for pH calibration have been envisioned: (1) pH Correction and (2) pH Calibration;

3) By selection pH Calibration proceed to the pH calibration menu;

4) Enter the buffer solution temperature in the pH calibration window by clicking on the digits (which are indicated under the title “Enter buffer temperature”) (see Figure 12.4);

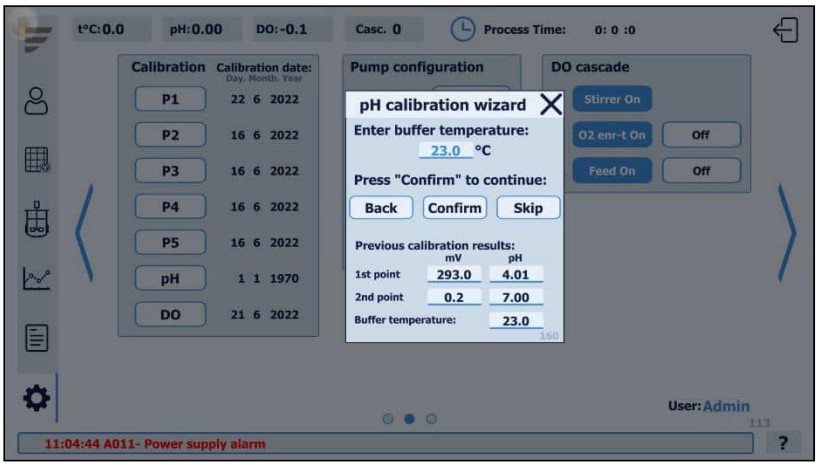

Figure 12.4. Buffer temperature entry window

5) The user has three option to navigate through the pH calibration wizard: Back, Confirm and Skip. By pressing the Back button the user can return to the previous calibration window (all entered data will be saved). By pressing the Confirm button the user can proceed to the next calibration window. By pressing the Skip button the user can skip the current calibration window (without saving any new data) and proceed to the next calibration window.

6) After the correct buffer temperature has been entered press Confirm;

7) In the next window, it is required to enter the first buffer solution pH value (see Figure 12.5);

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.

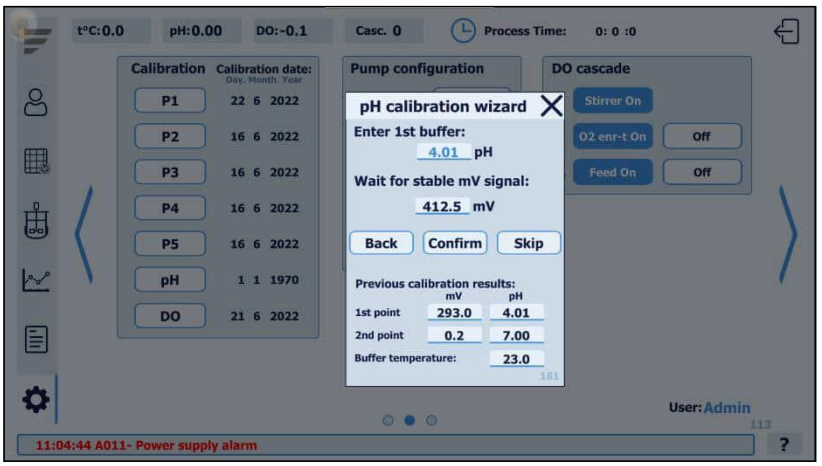

Figure 12.5. pH calibration wizard for calibration of the first buffer solution

8) Immerse the sensor into the buffer solution, enter its pH value and wait until the mV readings stabilize;

9) When the mV value has stabilized - press Confirm;

10) pH calibration wizard for entering the second buffer solution's pH value will appear (see Figure 12.6);

11) For the second point calibration it is recommended to use buffer solution with a pH value that is close to the isopotential point (~7 pH);

Figure 12.6. pH calibration wizard for calibration of the second buffer solution

12) Immerse the sensor into the second buffer solution, enter its pH value and wait until the mV readings stabilize. When mV value has stabilized – click Confirm;

13) After pressing Confirm a notification will appear, where the new and old calibration data is displayed. Additionally, the actual (measured) pH value, in accordance to the new calibration data, is displayed (see Figure 11.5);

14) To save the new calibration data press the Save button.

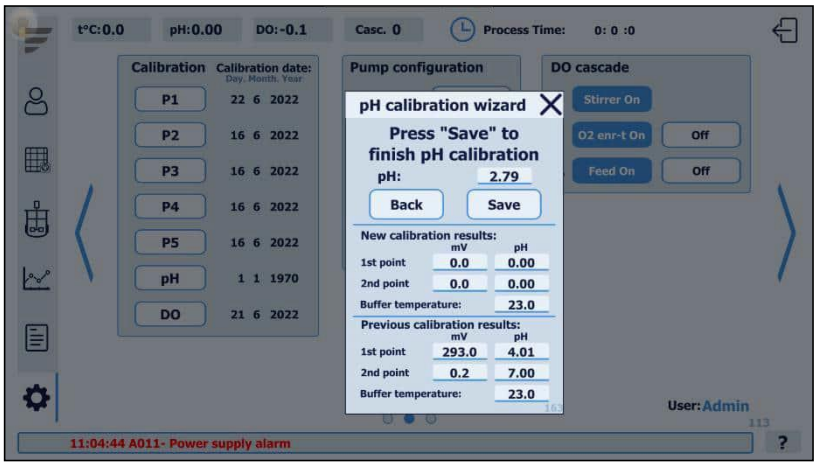

Figure 12.7 pH calibration wizard with completed calibration

15) By selection pH Correction function the user can access the correction wizard, where it is possible to re-calibrate the sensor during a running cultivation with the help of a reference electrode (see Figure 12.8);

Warning!

Upon entering the pH correction wizard the on-line monitoring of pH is suspended. The user is advised to access the mentioned window while taking the medium sample, which will be used for reference measurements.

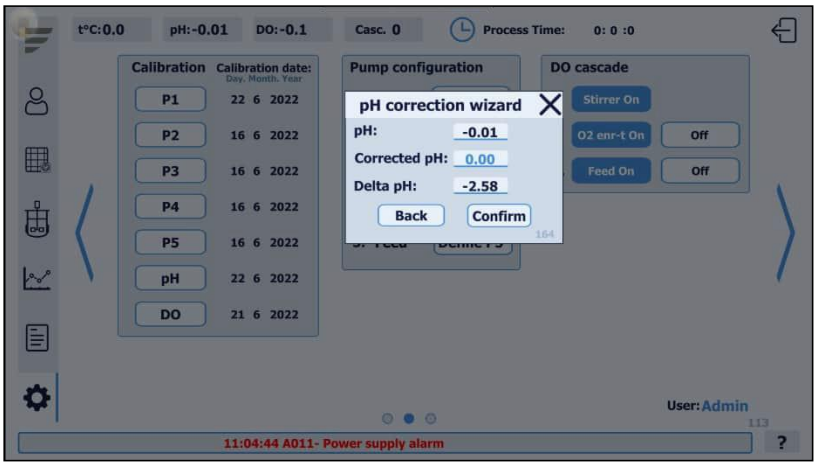

Figure 12.8. pH correction wizard

16) Take a sample of the cultivation medium. Place a reference pH electrode into the sample and measure the pH of the solution;

17) Enter the pH value measured by the reference electrode into the pH correction wizard.

18) By pressing the Confirm button the pH correction procedure is finalized (see Figure 12.9).

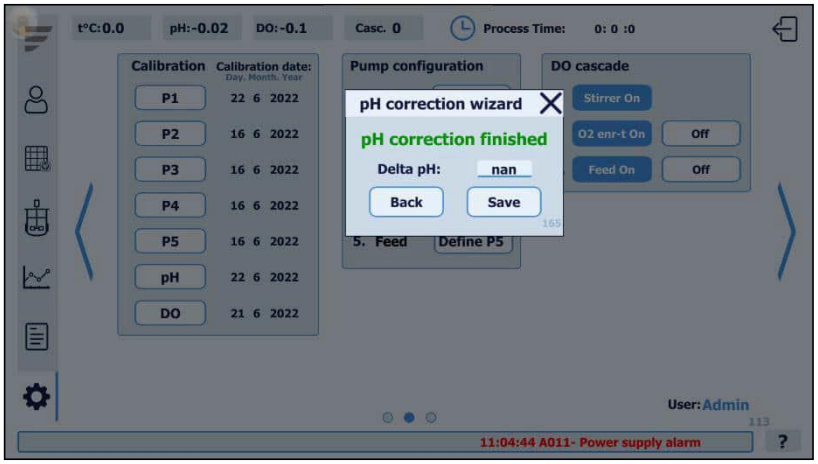

Figure 12.9. pH correction finished

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).

13. 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 BioReactor system, DO can be controlled via automatically manipulated variable of: (1) stirrer rotation speed, (2) inlet air enrichment rate of O_2 flow (see Section 16: "Dissolved oxygen cascade control"), (3) and substrate feeding rate.

Notice!

Handling of the DO sensor has to be done according to the instructions described in Section 29.2: "MAINTENANCE AND STORAGE OF THE pO2 SENSOR".

To access the DO control window, open the REACTOR window, locate the DO section (highlighted in Figure 13.1) where the SP value can be set.

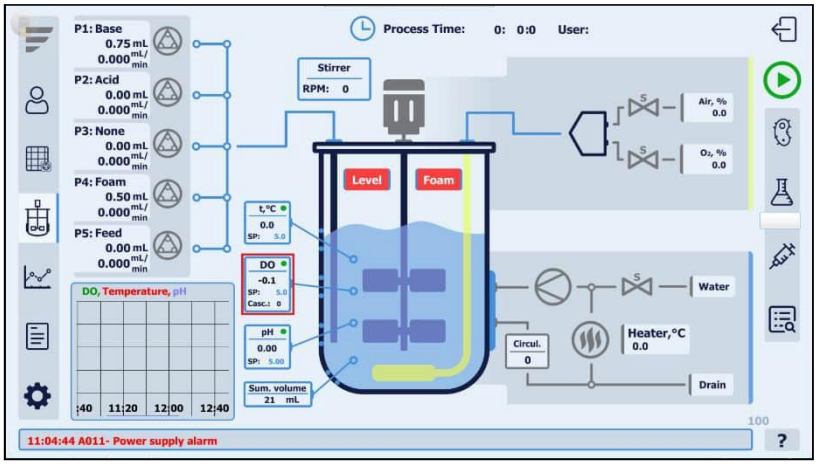

Figure 13.1. Window for setting the processes DO value

Or use the following access path: MENU → SETTINGS (1st WINDOW) → SETPOINTS to adjust the parameter set-point (see Figure 13.2).

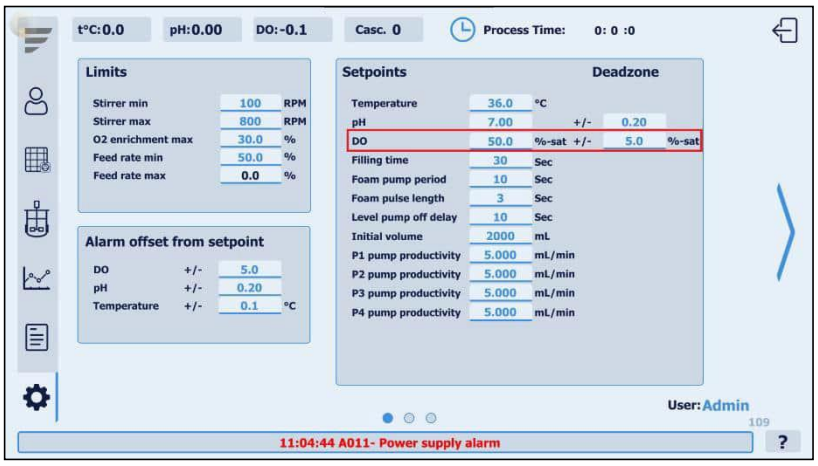

Figure 13.2. Window for setting the processes DO value in SETTINGS

Detailed information on process control setting selection, including DO control setting selection, is listed in Section 23.1: "CONTROLLER SETTINGS FOR STARTING THE PROCESS".

13.1 GENERAL INFORMATION ABOUTTHE DO SENSOR'S CALIBRATION

In general, DO 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 in two ways, i.e. using (1) two point or (2) one point linearization. While performing the upper mentioned actions, the sensor has to be subjected to either oxygen saturated and/or oxygen-free environments. Two point calibration is recommended when the sensor is being calibrated for the first time. Occasional two point calibration is recommended to identify any measurement errors, which can occur while using the sensor.

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 600 rpm and 2 – 3 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 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 500 rpm and 2 – 3 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.

Notice!

We recommend registering every calibration procedure results, such 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.

13.2 TWO POINT CALIBRATION

0 % point calibration

Choose one of the methods described in Section 13.1: "GENERAL INFORMATION ABOUT THE DO SENSOR'S CALIBRATION" – "Ensuring oxygen-free environment" and follow the instructions listed below to carry out the DO sensor calibration.

1) Use the access path MENU → SETTINGS (2nd WINDOW) → CALIBRATION → DO, to open the DO calibration wizard (see Figure 13.3) and select the desired calibration method;

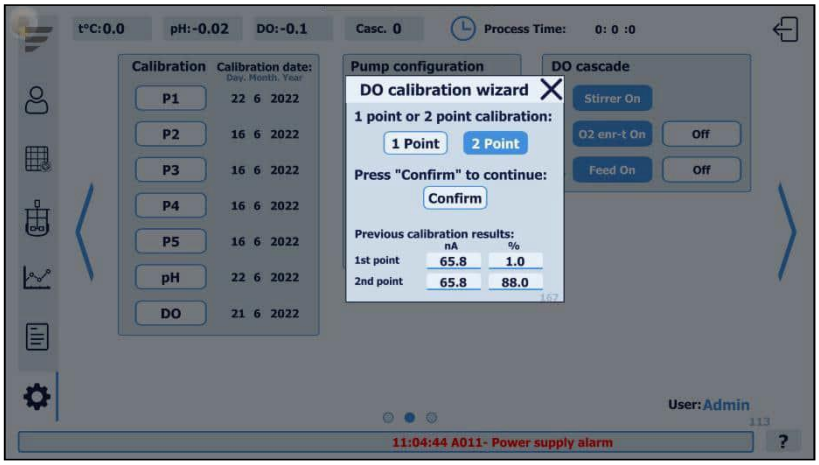

Figure 13.3. DO calibration wizard

2) Choose 2 Point, then - press Confirm. Notification with instructions for placing the electrode in an oxygen-free environment will appear, enter the value of the first calibration point (0 %) (see Figure 13.4);

Warning!

To ensure correct and precise two point calibration, always start with 0 % point calibration.

3) The user has three option to navigate through the DO calibration wizard: Back, Confirm and Skip. By pressing the Back button the user can return to the previous calibration window (all entered data will be saved). By pressing the Confirm button the user can proceed to the next calibration window. By pressing the Skip button the user can skip the current calibration window (without saving any new data) and proceed to the next calibration window.

4) Subject the electrode to an oxygen-free environment and wait for the nA reading to stabilize (usual in the range of 0 – 1.5 nA), see Figure 13.4;

5) Press Confirm, this will bring up the next calibration wizard with instructions saying to immerse the electrode in an oxygen-saturated environment (100 % point calibration), see Figure 13.5.

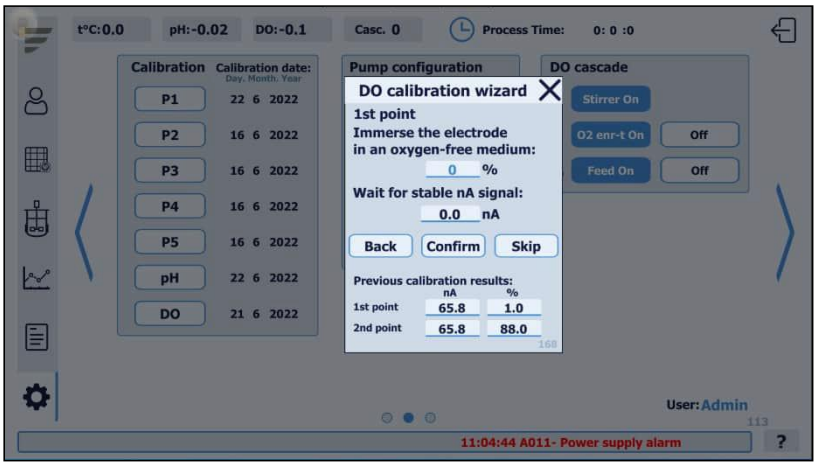

Figure 13.4. Two point DO calibration wizard (first step)

100 % point calibration

Choose one of the methods described in Section 13.1: "GENERAL INFORMATION ABOUT THE DO SENSOR'S CALIBRATION" – "Ensuring oxygen-saturated environment" and follow the instructions listed below to carry out the DO sensor calibration.

1) Subject the electrode to an oxygen–saturated environment and wait for the nA reading to stabilize (usual in the range of 50 – 80 nA) (see Figure 13.5);
2) After pressing Confirm a notification will appear, where the new and old calibration data is displayed. Additionally, the actual (measured) DO value, in accordance to the new calibration data, is displayed (see Figure 13.6);
3) To save the new calibration data press the Save button.

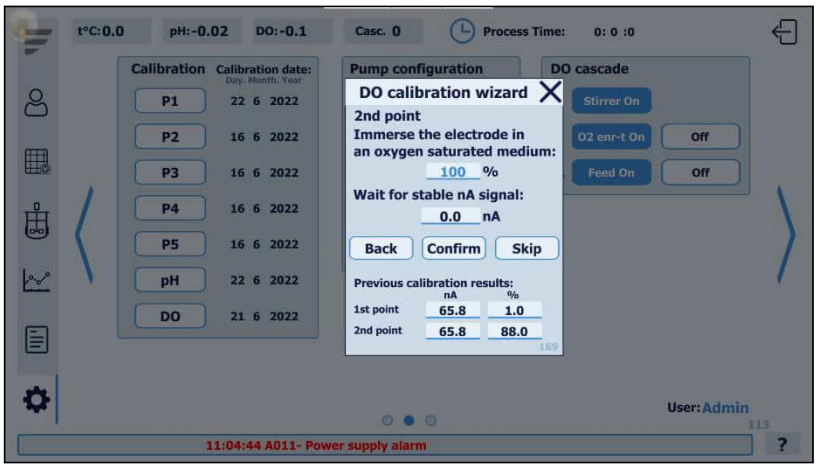

Figure 13.5. Two point DO calibration wizard (second step)

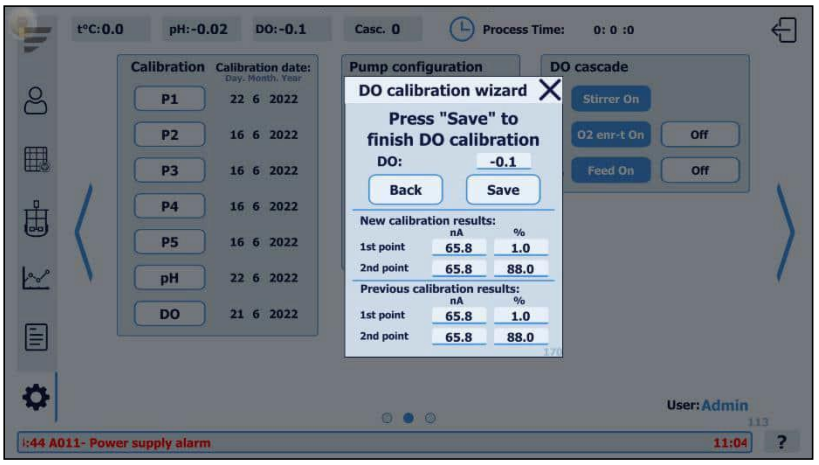

Figure 13.6. DO calibration finished

13.3 ONE POINT CALIBRATION

100 % point calibration

Choose one of the methods described in Section 13.1: "GENERAL INFORMATION ABOUT THE DO SENSOR'S CALIBRATION" – "Ensuring oxygen-saturated environment" and follow the instructions listed below to carry out the DO sensor calibration:

1) Use the access path MENU → SETTINGS (2nd WINDOW) → CALIBRATION → DO to open the DO calibration wizard (see Figure 13.3);
2) Choose 1 Point, then - press Confirm;
3) Perform steps 2-4 that are described in Section 13.2: "TWO POINT CALIBRATION" – 100 % point calibration.

14. 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 bioprocess controller when the foam rises to a pre-set level. If the sensor signal becomes active; within the REACTOR window the Foam alarm is signalized. After the bioprocess controller 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.

14.1 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 5 – 10 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 (1st WINDOW) → SETPOINTS (see Figure 14.1).

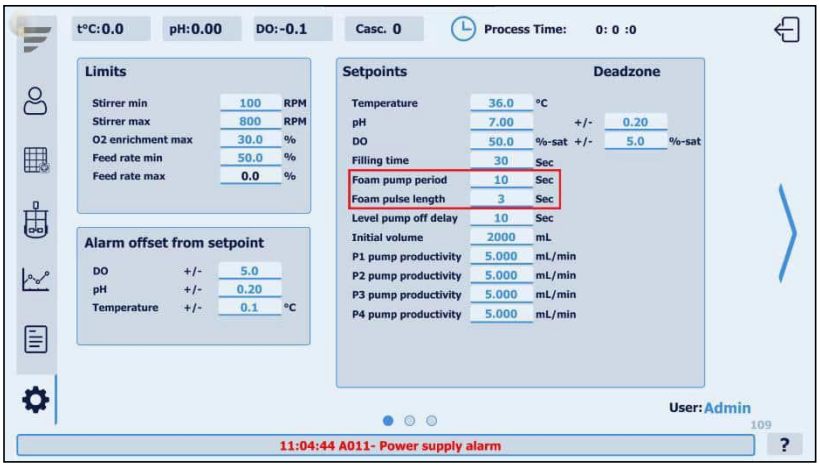

Figure 14.1. Foam pump period settings

The user can define both the Foam pump period and Foam pulse length. The respective pump, upon receiving a signal from the bioprocess controller will operate in pulses, where each pulse time is equal to the Foam pulse length and the pause between pulses is equal to the difference between the Foam pulse length and the Foam pump period, e.g. in the given example the pump will operate for 3 seconds after each 7 seconds.

15. Level control

Level regulation is carried out in a similar way as in the case of foam level control, i.e. by the conductivity sensor signal. If the sensor signal becomes active; within the REACTOR window the Level alarm is signalized. Reaction medium will be pumped out of the bioreactor through a chemostat tube. A respective port has to be mounted on the upper bioreactor lid for level control implementation. The end of the chemostat tube has to be submerged in the reaction medium.

15.1 LEVEL CONTROL PRINCIPLE

The foam sensor should be installed according to the peculiarities of each specific process. The BioReactor system allows a time delay to be set for the level control algorithm. The level pump delay is applied in the time period between the activation of the level signal and the activation of the LEVEL pump. To set-up the level pump off delay, use the access path: MENU → SETTINGS (1st WINDOW) → SETPOINTS (see Figure 15.1).

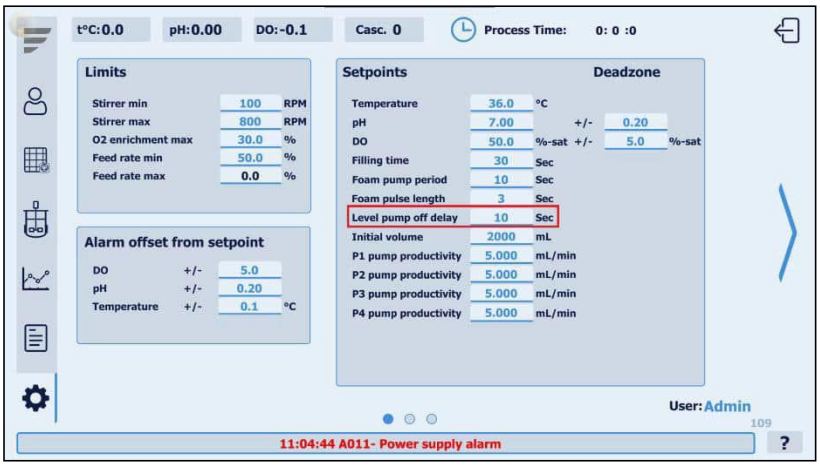

Figure 15.1. Level pump off delay settings

16. Dissolved oxygen cascade control

In the BioReactor system, DO can be controlled via automatically manipulated variables (cascades) of (1) stirrer rotation speed, (2) inlet air enrichment rate of O_2 flow, (3) and substrate feeding rate.

16.1 THE PRINCIPLES OF ESTABLISHING THE DO CASCADE CONTROL

The general DO cascade control principles are described further. At first, the SP (Set Point) and DZ (Dead Zone) are set for the necessary DO control range. The respective performance mechanism works on the process in such a way that the DO value is maintained within the range of SP - DZ < DO < SP + DZ. If the DO value is within the aforementioned range, no control action of manipulated variable takes place. If DO value is beyond such range, the manipulated variable is being controlled according to the PID algorithm. In all cases, except for the oxygen enrichment impulse control, the manipulated variable is controlled by a proportional control signal (between 4 - 20 mA or 0 – 10 V). The regulation is suspended in each cascade when the minimum or maximum limiting value has been reached. The control of all of the aforementioned variables can be combined into DO cascade control.

DO control may take place with different cascades. The control of each cascade takes place according to the described types. Any of the described cascades can be selected and any order for their sequencing can be established.

Cascade control is working in line with the following principles:

1) Appropriate cascade sequence of manipulated variables should be defined before process. The particular manipulated variable control happens until upper limit of its set-point or lower limit for cascades no. 2 – 3 is reached. When the limiting set-point is reached, the transition to the next or previous cascade takes place depending on which (maximum or minimum) limiting value has been reached. Transition to the next cascade cannot take place if the current cascade is the last one;
2) Regulation begins in a specific cascade when a certain DO and manipulated variable limits is reached. When a limiting value has been reached, the transition to the next or previous cascade will take place. The manipulated variable continues in operation in the following cascade with its preceding status. The only exception is when the following cascade is "substrate feeding". In such an event the cascade begins with the productivity of the feeding pump, which was actual from the particular feeding profile. If feeding was not active according to the feeding profile, then feeding shall begin in this cascade with some of the limiting values (minimum or maximum in percents from feeding pump's maximal productivity).

16.2 DO CASCADE CONTROL

Access DO cascade control by using the path: MENU → SETTINGS (2^{nd} WINDOW) → DO CASCADE.

Figure 16.1. PUMPS and DO CASCADE control window

The given window offers in DO cascade control used manipulated variable of Stirrer speed, O_2 enrichment and Feedrate, configuration (see Figure 16.1). To choose manipulated variable order use the respective buttons and select appropriate cascade performance element.

17. Process trends

To open the trends menu use the access path MENU → TRENDS, see Figure 17.1.

Figure 17.1. TRENDS window

The TRENDS window displays the changes in the dynamics of process parameters (temperature, pH, DO, stirrer, pumps, etc.), by clicking on the maximize buttons for the 1st and 2nd trend fields the user can gain access to a larger window, which displays the respective trends (see).

Within each trend window the user can:

- Configure the scale of the Y axis for each individual parameter (see Figure 17.2);
- Change the visibility of each parameter (see Figure 17.4) accessed by pressing the cog button on the Trend field (see Figure 17.3).
- Change the visibility of the Y axis for each individual parameter (see Figure 17.5) accessed by pressing the cog button on the Trend field (see Figure 17.3).

Figure 17.2. Y axis scale configuration window

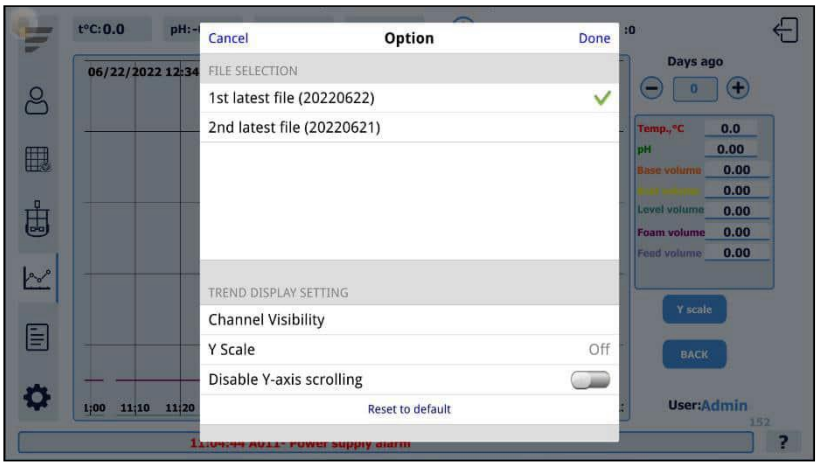

Figure 17.3. Trend configuration menu

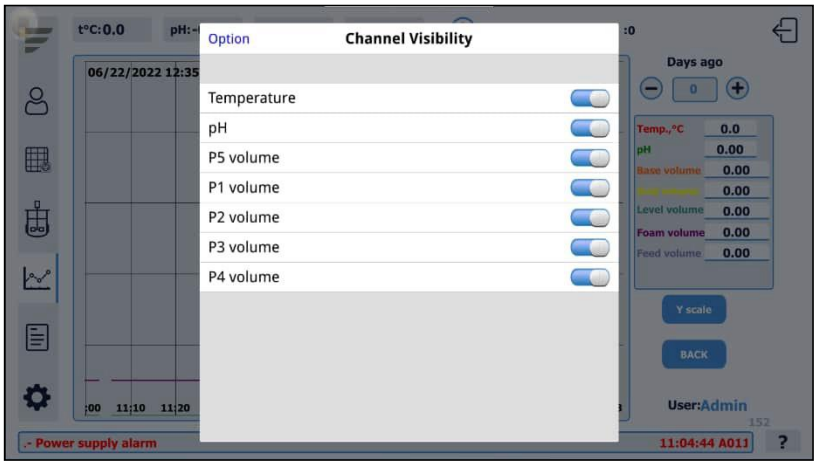

Figure 17.4. Trend visibility settings

Figure 17.5. Trend Y axis visibility settings

Additionally, the on-line graphical view of the main process parameters are displayed within the REACTOR window (highlighted in Figure 17.6).

Notice!

The user can navigate through the trend field by dragging the image left and right. Additionally it is possible to magnify a particular region by spreading the image using two fingers.

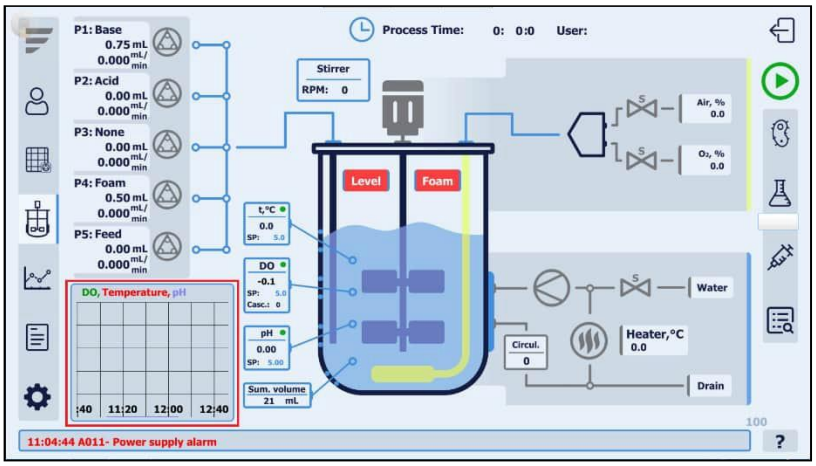

Figure 17.6. Trend section within the REACTOR window

18. Alarms and events

18.1 SYSTEMALARMMESSAGES

To access the alarm off set settings use the following access path MENU → SETTINGS (1st WINDOW)
→ ALARM OFFSET FROM SETPOINT, see Figure 18.1.

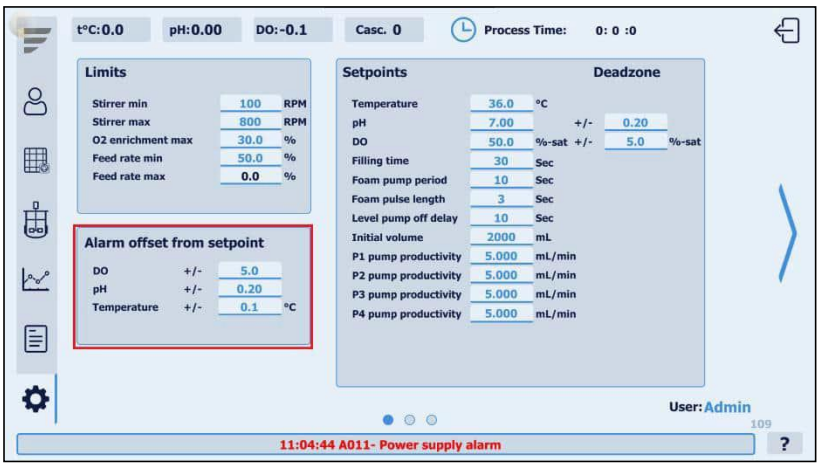

Figure 18.1. ALARM OFFSET window

The alarm offset window allows setting-up Low and High alarm limits for temperature, pH and DO. An alarm is triggered as soon as the process value drops below or goes over the pre-set limit - the alarm notification appears in red text in the bottom of the display, e.g. see Figure 18.2.

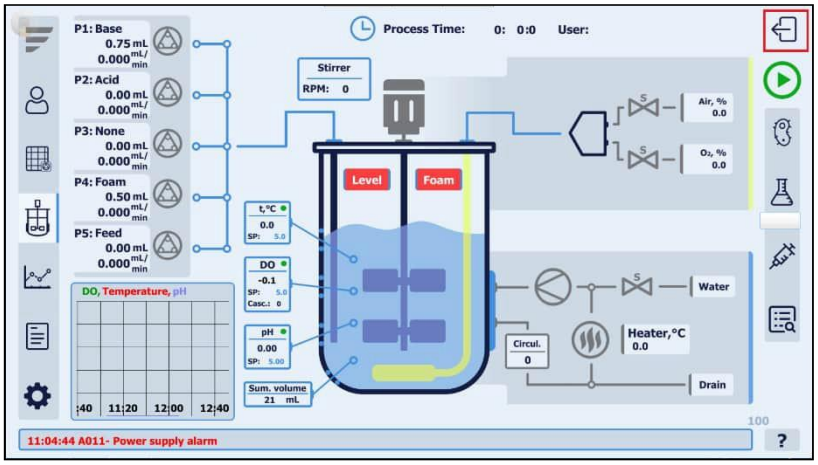

Figure 18.2. REACTOR window with an alarm notification at the bottom of the display

The information about the time of the event, cause of the event and date is displayed in the alarm notification.

If the reason for the alarm is resolved (e.g. during regulation the parameter already is within the required limits or the operator has switched off the alarm signal), the text disappears.

List of alarm notifications (Figure 18.3) can be found by using the access path MENU → ADMINISTRATION → ALARMS.

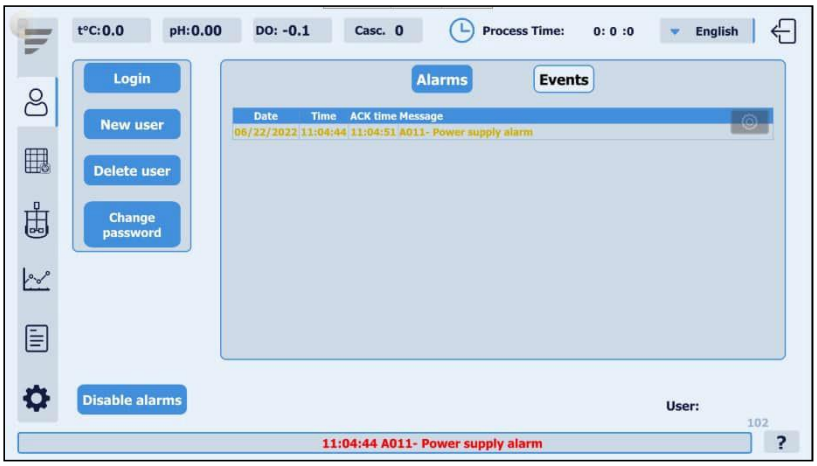

Figure 18.3. ALARM notification window

By using the access path MENU → ADMINISTRATION → DISABLE ALARMS, it is possible to open the list of alarms, where it is possible to mark the alarms, for which upon triggering a notification will be displayed in the bioprocess controller, see Figure 18.4.

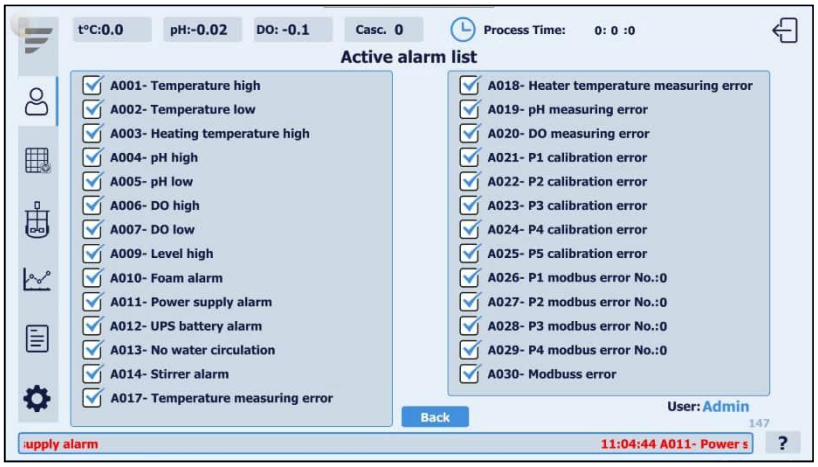

Figure 18.4. Active alarm list window

18.2 SYSTEMEVENTS

List of event notifications (see Figure 18.5) can be found by using the access path MENU → ADMINISTRATION → EVENTS.

In the EVENTS window the user can gain access to a database, which displays a list of all actions, which were performed with the system in a chronological order. Additionally, information on the previously active user, event type, equipment state etc. is provided within the mentioned window.

Figure 18.5. EVENT HISTORY window

19. PID parameters of process control

Bioprocess controller is applied to ensure precise regulation of process parameters. Their control software work according to the proportional-integral-derivative (PID) algorithms.

Such algorithms determine the extent of processes control signal effect on the performing mechanism. The regulation signal magnitude is calculated with the following equation:

$$u(t) = Pe(t) + I\int_0^t e(\tau)d\tau + D\frac{de(t)}{dt}$$

where,

u(t) – extent of effect in time *t*;

e(t) – error, i.e. difference between the set-point (SP) and the process value (PV);

P,I,D – proportional, integral and derivative ratio, respectively.

The proportional P ratio determines the effect directly proportional to *e(t)*. The larger is P, the more sensitive is the effect. If P is too large, the regulation may de-stabilize due to intensified oscillations. If P is too small, the regulated parameter reaches the set-point value (SP) too slowly or may fail to reach it at all. Using only the proportional P ratio, in most cases sufficient preciseness of regulation cannot be achieved.

The integral ratio I determines the effect considering the mean summary error in the specific time. As a result, regulation becomes more stable and advanced. The set-point value (SP) of the regulated parameter is being reached faster.

The derivative ratio D determines the effect of the control signal considering the speed of change of the regulated parameter. For example, if the temperature set-point during the regulation process SP = 37 °C and PV = 35 °C, the algorithm determined the magnitude of the control signals output. The respective control elements implement the regulation, the temperature reaches the set-point value in a specific amount of time. If the situation will be similar, but the amount of time needed for the parameter to reach the set-point value will differ, the D parameter comes into place and corrects the output control signals magnitude. The P and I ratios do not consider the dynamics of value changes, and therefore correct setting of the D parameter is of utmost importance for dynamic processes to ensure sufficient preciseness of regulation.

In addition to the aforementioned ratios the following parameters can be set in the PID parameter window:

Period - determines the time delay after which the changes of regulating effect take place. Such parameter is working with considering sensor and process momentum.

Time lag - time lag of the derivative action. The algorithm of the D action includes a time lag that can be assigned at the “time lag of the derivative action” input.

19.1 SETTING UP PID PARAMETERS

Open the PID settings window by using the access path MENU → SETTINGS (3rd WINDOW) (see Figure 19.1).

Figure 19.1. SETTINGS window (PID)

PID parameter coefficients for heating, cooling, DO stirrer, DO feed and DO enrich.

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.

The BioReactor system provides options for fine-tuning the PID control algorithms in respect to the peculiarities of different cultivation/fermentation processes. The user can enable auto tuning of PID coefficients during a running fermentation for the following process parameters: DO control by stirrer, DO control by O_2 enrichment and DO control by feeding.

In order to enable the auto tuning procedure press the Fine tuning button in the respective PID coefficient section and then press the Start tuning button (see Figure 19.2). After enabling fine tuning the user can terminate the procedure at any time by pressing the Stop tuning button. If the procedure is terminated prior reaching the end-state, the new PID coefficients will not be saved to the system. The auto tuning procedure is finished automatically., to access other PID parameters press buttons PID 1, PID 2 and PID 3 to navigate through the PID configuration window.

Warning!

During the auto tuning procedure minor fluctuations in the control of target process parameters can occur.

Figure 19.2. Window for auto tuning of DO control PID parameters

20. Feed control

The BioReactor system ensures feeding control by two options: by (1) feeding control via feeding rate vs. time profile and (2) DO concentration control.

20.1 TIME PROFILE OF FEEDING RATE

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

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

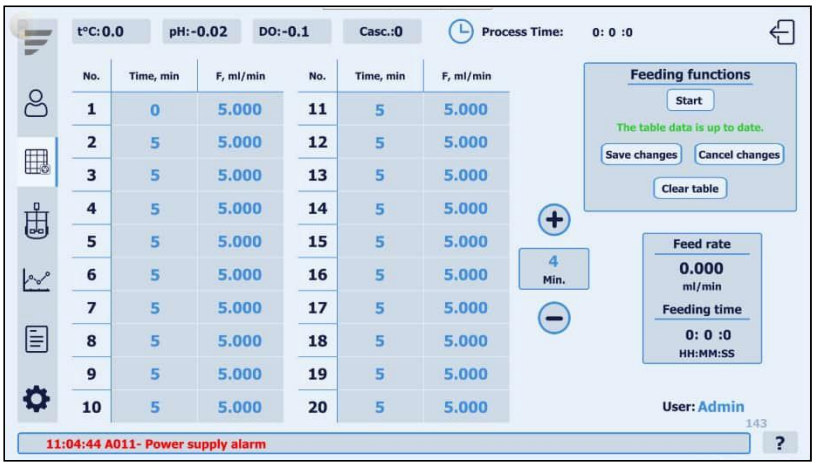

Figure 20.1. FEED window, TIME SHIFT pop-up window

Within the FEED window (Figure 20.1) it is possible to set-up 20 values of time and corresponding feeding rates. Time tags correspond to the fermentation process time.

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.

In the FEED window it is also possible to move the whole feeding profile either further or backwards in respect to the process time by using the Time Shift function.

Using time shift it is possible to change the time of the feeding profile time by subtracting or adding the necessary time (in minutes) to all feeding profile points. To do so, enter the desirable time and use buttons "-" to subtract or "+" to add the entered time to the profile points, see Figure 20.1.

Feeding is activated by pressing the START FEED button. Feeding rate will be activated depending on the defined process times and selected feeding profiles approximation. Feeding can be stopped using the same button that indicates STOP FEED while the feeding profile is active. When zero values are introduced into the feeding profile time, the feeding will be terminated despite if further in the profile different values are entered.

20.2 DISSOLVED OXYGEN(DO) CONTROL BY FEEDING RATE

Dissolved oxygen control is maintained at a pre-set level, by automatically adjusting the feeding (substrate addition) rate.

If the SP (Set Point) and DZ (Dead Zone) for the DO value are defined, the stirrer operates in such a way, to maintain the process DO value at: SP ± DZ. If the processes DO value (PV) is inside the dead zone corridor SP ± DZ, the PID output value is calculated, but feeding rate adjustments are not implemented(no action is taken). Feeding is carried out as defined by the feeding profile. The feeding rate control can be carried out only if the feeding is in progress. If the processes DO value (PV) is outside the dead zone corridor SP ± DZ, then the feeding rate is adjusted by the PID algorithm so that the PV returns into the dead zone corridor, the following algorithm is implemented based on the sign of the proportional pO_2 feed PID coefficient:

Feeding rate is decreased, if the PV < SP ± DZ. The minimum pumps' productivity is limited by the Feed rate min parameter(%). The Feed rate min parameter is defined by the user (highlighted in Figure 20.2).

Feeding rate is increased, if the PV > SP ± DZ. The maximum pumps' productivity is limited by the Feed rate max parameter (ml/min). The Feed rate max parameter is defined by the feeding profile.

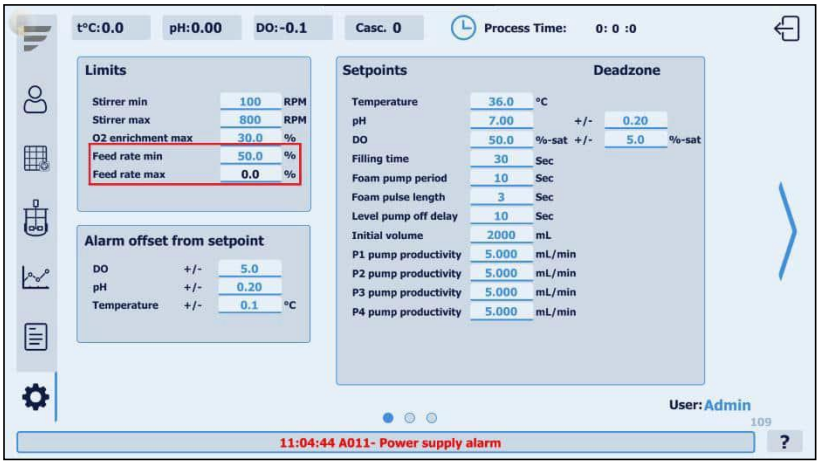

Figure 20.2. Feed profile parameters: Feed rate max and Feed rate min

21. 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 BioReactor system's software, bioreactor's tank, its constituents and other equipment;
2) Make sure that the bioreactor tank is located correctly in respect to the bioprocess controller and respective service connections (more detailed explanations in Section 4.1: "INSTALLATION OF THE BIOREACTOR VESSEL");
3) Make sure that all service connections have been carried out according to Section 4.4: "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 4.2: "DESCRIPTION OF THE REACTOR LID AND PORTS", Section 4.3: "SENSOR" and Section 4.5: "VESSEL CONNECTIONS";
6) 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;
7) Carry out sterilization of the bioreactor tank and its inputs (which will be in contact with the reaction environment) as described in Section 28: "Sterilization of the vessel";
8) 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.

21.1 ADDING THE REACTION ENVIRONMENT TO THE BIOREACTOR VESSEL

When all activities listed in Section 21: "Preparation for the process" have been carried out, the reaction environment can be supplied to the sterilized bioreactor vessel.

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 11: "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.).

After the fermentation medium has been introduced into the bioreactor the user can setup the initial medium volume setting by following the access path: MENU → SETTINGS (1st WINDOW) → SETPOINTS (see Figure 21.1).

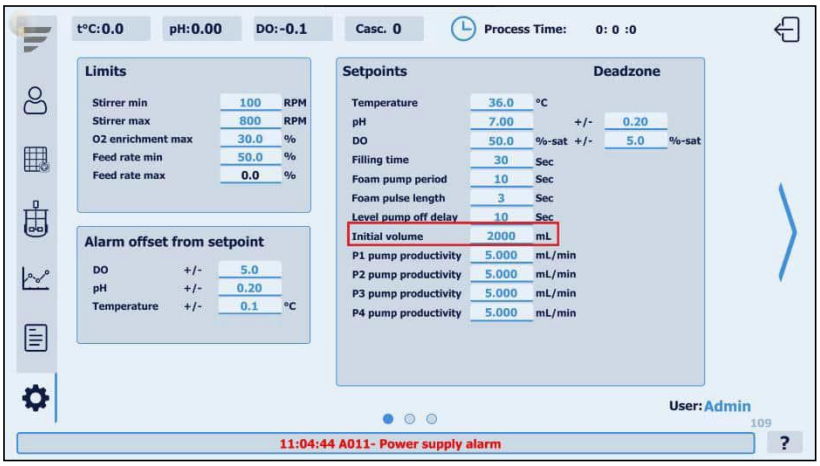

Figure 21.1. Initial medium volume setting

Notice!

By entering the precise medium volume value and defining the inoculum, sample and induction volumes it is possible to automatically log the total volume contained in the bioreactor during fermentation.

22. Recipes

Using the access path MENU → RECIPES it is possible to open the Recipes window where it is possible to configure, save, load to and copy recipes from the system (see Figure 22.1).

A Recipe in the BioReactor system is a set of parameter values (setpoints, limits, feeding profile etc.), which can be saved and stored in the system. Upon necessity the user can select particular recipes and upload them to the controller, which removes the need of inputting each set of parameter values by hand.

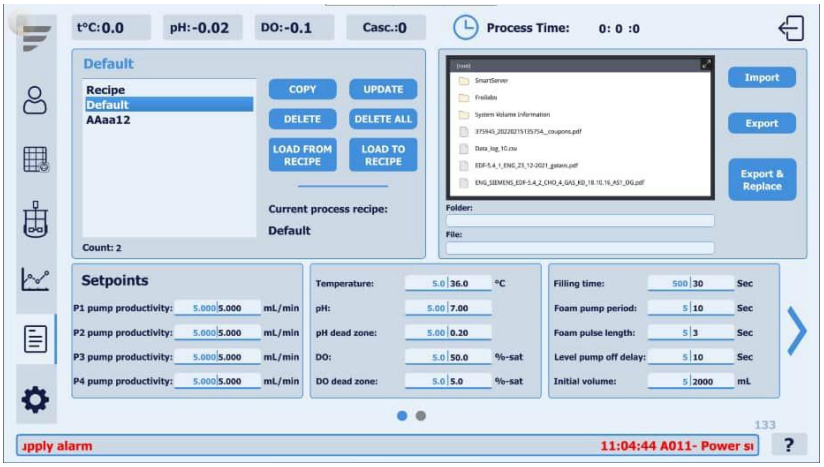

Figure 22.1. Recipe window

To create a new recipe enter the respective name (see Figure 22.2), and press the Copy button. A new recipe will be created and displayed in the list.

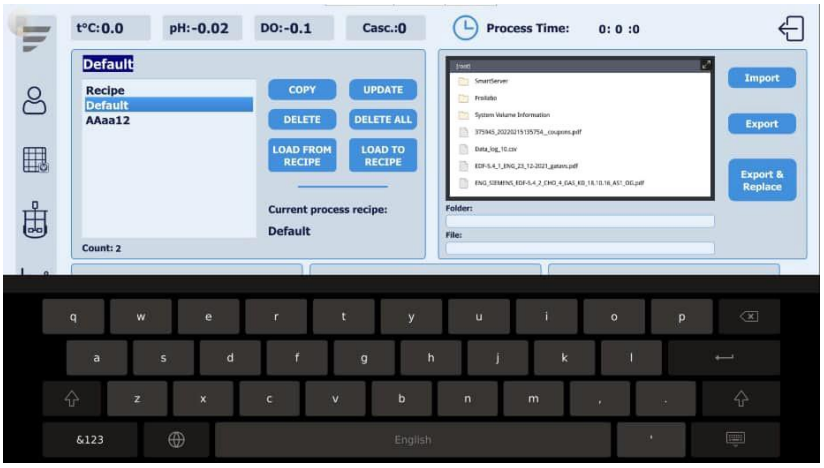

Figure 22.2. Creating a new recipe

The user can select recipes by clicking on the respective names, which are displayed in the list.

In the lower part of the recipe window the respective values of parameters are displayed (see). The digits in black represent the values, which are currently saved in the controller, e.g. are inputted into the BioReactor system. The value in blue, represent the data saved within a particular recipe and they can be modified by the user.

To modify a particular recipe, select it from the list and carry out changes of desired parameters. After all required changes have been carried out press the Update button.

Additionally, the user can copy all parameter values, which are currently inputted into the controller to a recipe by pressing the Load to recipe button.

To transfer the data, which is stored in a recipe to the controller press the Load from recipe button.

To delete a particular recipe press the Delete button.

To delete all recipes press the Delete all button.

For convenience, the user can store particular recipe sets, e.g. the whole list of recipes, which is displayed in the Recipe window, on a separate data storage device (USB flash drive).

To carry out the mentioned procedure:

1. Plug a USB flash drive into the back panel of the bioprocess controller;
2. Navigate to the desired directory of the flash drive by using the window on the left-hand side of the Recipe window;
3. Define a file name for the recipe set by inputting the name into the file field;
4. Press the Export or Export & Replace button.

To import a recipe set use the Import button.

23. Starting the process

Before enabling the process using the BioReactor system, instructions described in Section 21: "Preparation for the process" should be adapted as was previously described.

23.1 CONTROLLER SETTINGS FOR STARTING THE PROCESS

Before starting the process, carry out the following actions to set-up the bioprocess controller settings:

1) Set the control parameter values (SP), using the access path, MENU → SETTINGS (1st WINDOW) → SETPOINTS. Alternatively, navigate to the REACTOR window and change the set-points there for every desired control parameter;
2) Set the limits of control elements by using the access path: MENU → SETTINGS (1st WINDOW) → LIMITS;
3) 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!

23.1 STARTING THE PROCESS

To enable the process click the START button in the REACTOR window (see Figure 23.1).

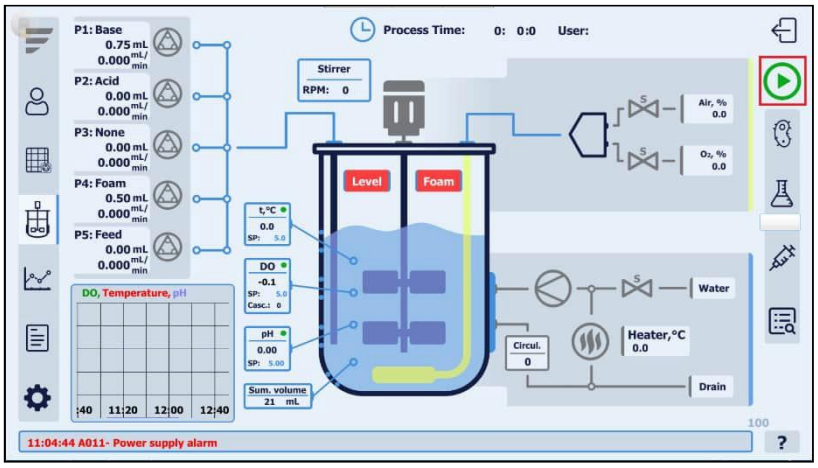

Figure 23.1. Window for starting the process

23.2 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 the septa port. In all cases the activities have to be carried out in line with the instructions described in Section 21.1: "ADDING THE REACTION ENVIRONMENT TO THE BIOREACTOR VESSEL".

To eliminate the processes 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 manual or automatic temperature control of environment inside the bioreactor (see Section 7.1: “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.

After inoculation the user can specify the added volume of seed material to the fermentation medium in the REACTOR window (see Figure 23.2). The mentioned value upon entering will be taken into account during automatic logging of the medium volume.

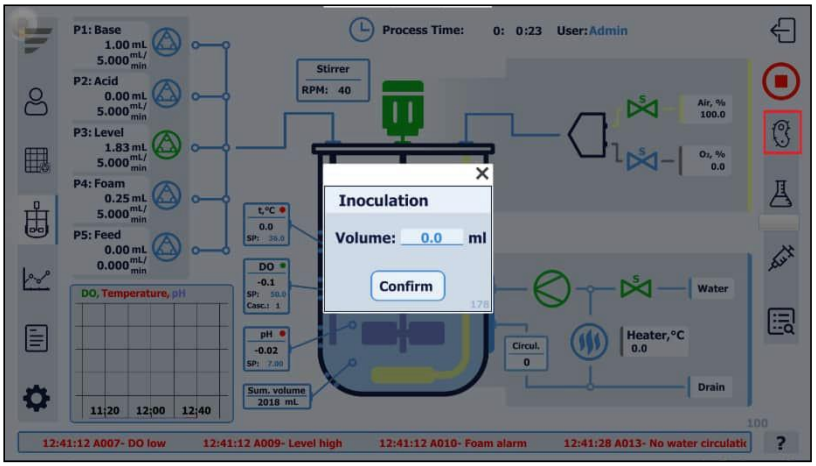

Figure 23.2. Inoculum volume window

24. Process control with iOS/Android tablets and smartphones

The option for remote (WiFi) monitoring and control of the fermentation process using *iOS* or *Android* tablets and smartphones has been provided. The connection to the bioprocess controller is established via built-in router into the BIO-4 controller.

The *iTeleport* application should be installed on your device to gain access to the upper mentioned functions. Upon configuring and connecting to the BIO-4 bioprocess controller, you will acquire a visually and functionally identical copy of the bioprocess controller's display in your *iTeleport* app.

25. Sampling, induction 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 BioReactor system are described below.

Both sampling and harvesting actions can be performed through the lower port of the vessel - port (1) and port (2). For sampling it is common to use one of these ports and to close the other one with a specified needle port plug. To start sampling or harvesting, open the tap by screwing it clockwise (see Figure 25.1)

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 1 - 20 mL.

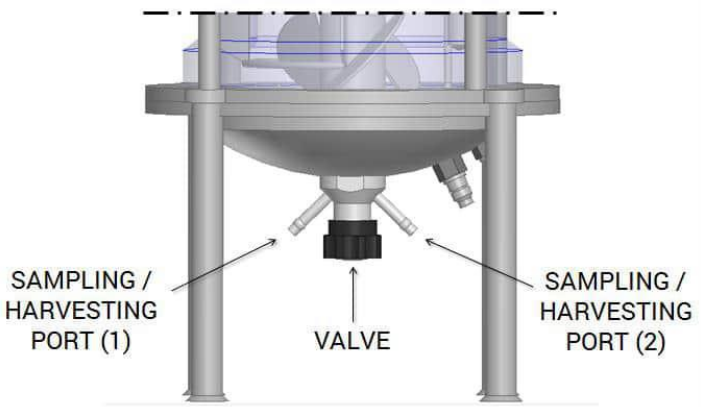

Figure 25.1. Sampling port

25.1 SAMPLING AND SAMPLING CHANNELSTERILIZATION

In the BioReactor system an option to sterilize the sampling channel with steam is provided. Steam sterilized sampling line is shown in Figure 25.2.

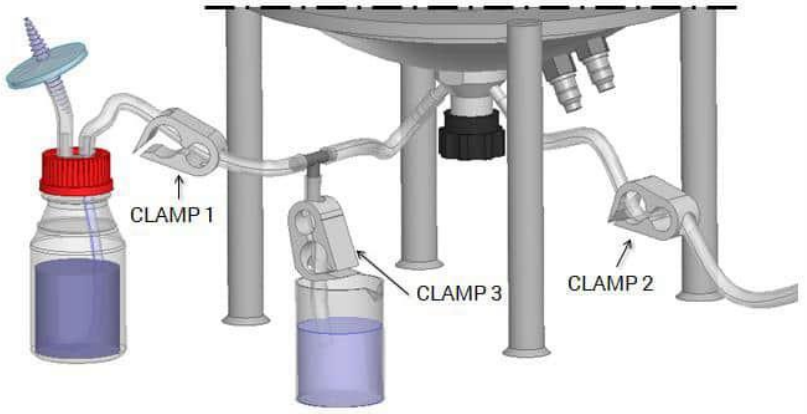

Figure 25.2. Sampling channel sterilization

In Figure 25.2 port (2) is used for the steam supply. The sterilization procedure is performed as follows:

1) Connect port (2) to the steam supply via temperature resistant silicone tube with an installed clamp (2);
2) Connect a short silicone tube to port (1). Connect the other end of the tube to a T-shaped divider;
3) Connect a short silicon hose with a clamp (3) to one end of the divider and place the end of the tube into a beaker filled with 70 % ethanol;
4) Connect a longer silicone hose with a clamp (1) to the second end of the divider and connect it to a small bottle filled with deionized water;
5) Sterilize the sampling channel by opening the clamps (1) and (2), and open the steam supply. Let the steam flow through the channel for about 1 – 2 min to sterilize the sampling line;
6) After sterilization has been carried out, close the clamps (1) and (2);
7) Take out the tube that is immersed in ethanol out of the beaker and put its end into the sample container. Open the bioreactor tap afterwards;
8) Open the clamp (3) and collect the necessary sample volume into the container, see Figure 25.3;

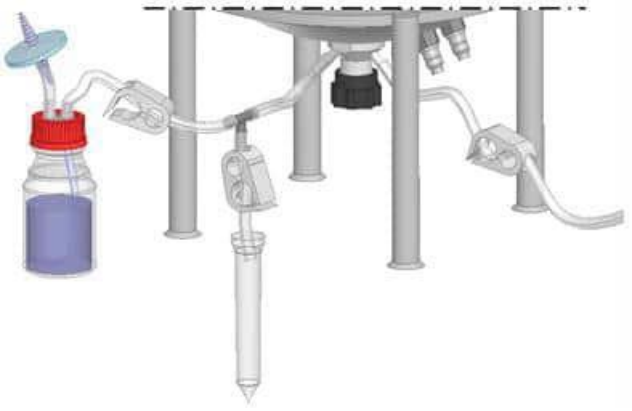

Figure 25.3. Sampling

9) After the sampling procedure has been carried out, close the clamp (3) and immerse the tube back into the beaker;
10) Close the tap.

Warning!

In order to avoid contamination during sampling or harvesting procedures and perform channel sterilization safely, it is recommended to strictly follow the instructions described in Section 25.1: “SAMPLING AND SAMPLING CHANNEL STERILIZATION”.

Notice!

If the sampling channel has not been sterilized prior the sampling procedure, in order to obtain a homogenous sample, it is recommended to discard a little amount of fermentation solution from the sampling channel (residue of the previous sample) before sampling.

After taking a sample the user can specify the substracted volume of the medium to the in the REACTOR window (see Figure 25.4). The mentioned value upon entering will be taken into account during automatic logging of the medium volume.

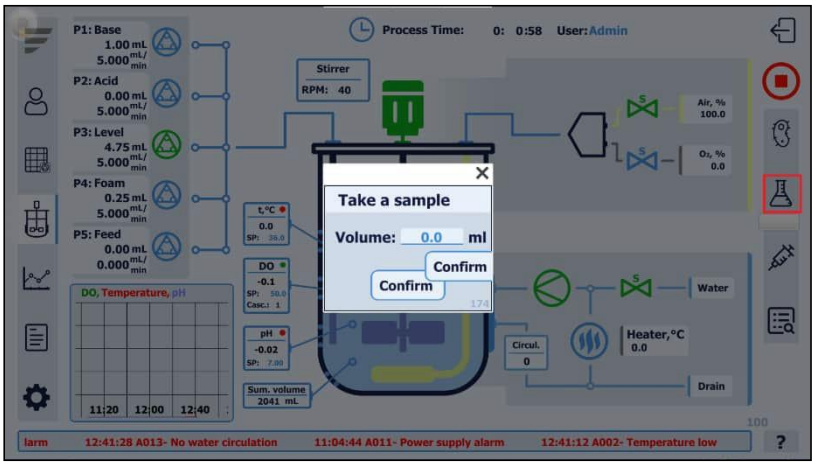

Figure 25.4. Sample volume window

25.2 INDUCTION

Inductor agents and/or other substances can be added to the reaction medium via peristaltic pump or manually, by supplying the respective solution via septa port (*optional*) or through one of the free inlets located on the vessels upper lid.

After adding an inductor to the medium the user can specify the added volume in the REACTOR window (see Figure 25.5). The mentioned value upon entering will be taken into account during automatic logging of the medium volume.

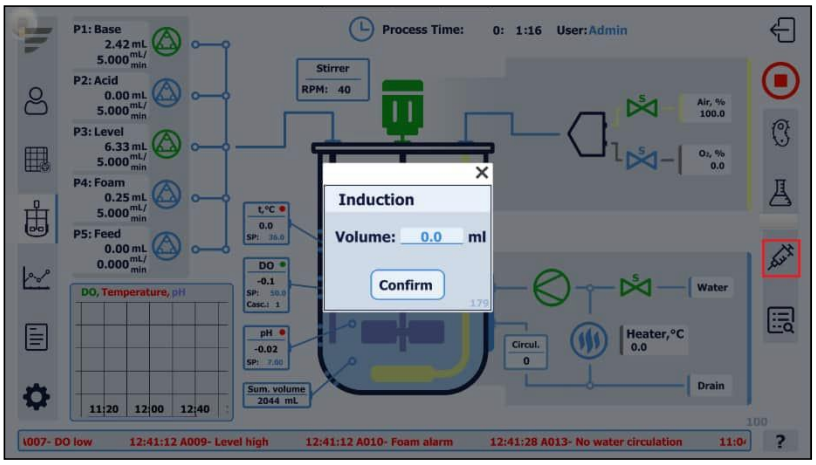

Figure 25.5. Inductor volume window

25.3 HARVESTING

In order to perform sterile harvesting of the entire bioreactor environment at the end of the fermentation process, perform the activities described further:

1) Prior harvesting, a sterile bottle or vessel should be prepared. Harvest bottle (large enough to hold the entire culture that is in the bioreactor vessel), should be equipped with a harvesting tube and a sterile filter for air exchange. The harvesting tube has to be long enough to reach from the bottle (placed lower than the vessel) to the lower part of the bioreactor's vessel where the sampling/harvesting port is located (see Figure 25.1);
2) When everything is prepared for the harvesting procedure, connect the harvesting tube to the sampling channel in aseptic conditions see Figure 25.2;
3) Open the bioreactor's tap (see Figure 25.1);
4) Open the clamp (3) (see Figure 25.2) and wait until the entire fermentation environment has been drained into the bottle;
5) Close the bioreactor tap and the clamp (3);
6) Aseptically disconnect harvesting tube from the sampling tube.

26. Completion of the process

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

For safety purposes, control elements (cooling valve, heater, stirrer, pumps, air valve and O_2 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!

27. 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 29: "Maintenance", to ensure appropriate maintenance and storage of the pH and pO_2 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 4.1: "INSTALLATION OF THE BIOREACTOR VESSEL".

Bioreactor turbine removal and cleaning is highly recommended after every fermentation process, see instructions on Section 4.2: "DESCRIPTION OF THE REACTOR LID AND PORTS".

28. 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!

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".

29. 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.

29.1 MAINTENANCE ANDSTORAGE OF THE pH SENSOR

After receiving the sensor, carefully remove all packaging, cut off the protective rubber layer from the sensors upper membrane, if such is supplied. Wash the sensor with distilled water and wipe it dry it 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.

29.2 MAINTENANCE ANDSTORAGE 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 pO_2 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.

29.3 STIRRER MAGNETIC SHAFT MAINTENANCE

Periodic lubrication of the stirrer magnetic shaft bearings is recommended, to extend the shaft's lifetime. Magnetic shaft's maintenance after 1-2 years or 6 000 operation hours is recommended. Please, follow the instructions listed below (see Figure 29.1) to perform the upper mentioned procedure.

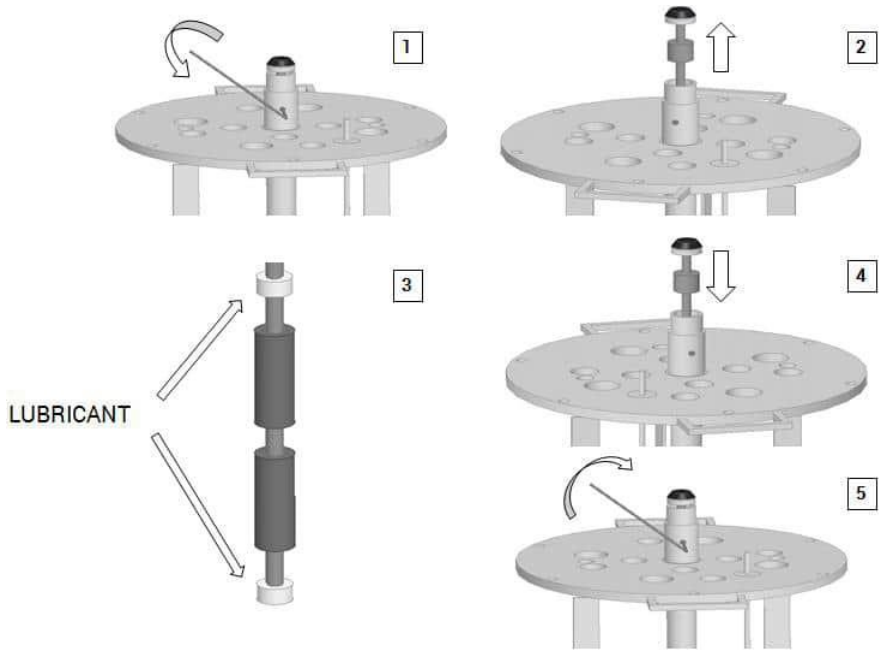

Figure 29.1. Illustrations of the magnetic shaft maintenance procedure

1) Using one of the supplied wrenches, loosen the screw located on the upper part of the stirrer shaft;
2) Carefully pull out the inner magnetic shaft;
3) Add the supplied grease on both shafts bearings. The amount of grease should be enough to cover the upper part of the bearing, we advise to put grease in an about 1 mm thick layer. The supplied grease is recommended for lubrication of metallic parts, which are subjected to diminish in high temperatures;
4) After adding the grease, carefully insert the inner magnetic shaft back into the outer agitator shaft. To maintain the appropriate magnet position, it is important insert magnetic shaft to its full extent;
5) Using the supplied wrench, firmly tighten the screw on the upper part of the stirrer shaft.

29.4 REACTOR VESSEL HERMETICITY TESTING

Periodic hermeticity test ensures that the vessel is completely hermetically sealed and ready for operation, Pressure leak could lead to vessel being infected by foreign microorganism. Most hermeticity leaks can be attributed to wear and tear, which can be addressed and corrected by using the supplied spare O-ring set and silicone sealant use for O-ring sealing. It is needed to perform the test on the vessel if there is a suspicion of an air leakage from the fermenter.

To test the hermeticity of the vessel all the ports on vessel should be plugged and hermetically sealed, lid should be tightly screwed on top and condenser exhaust port should be closed using the membrane valve. There should be no path for air to escape from the vessel.

Warning!

Never exceed pressure of 1 bar when testing for hermeticity!
Higher pressures could lead to glass tube cracking and exploding.

The test should be performed accordingly:

1) Make sure all paths for air to escape are closed with plugs or sensors Leave level and foam indicator in the vessel for hermeticity tests around the seals.
2) Attach air source to the air input port of the control cabinet (see Figure 4.9); make sure to adjust appropriate (not too high) air source pressure.
3) Set the pressure on manometer in front of the control cabinet in AIR section (see chapter 9, Air/O_2 supply settings, Figure 9.1).
4) In control panel close O_2 supply valve and open AIR valve, open the rotameter fully and wait for rotameter to show that there is no air flow in the line and all the air is now compressed in the vessel.
5) Close air AIR valve in control panel and wait for 10 minutes without changing the line pressure or performing any other parameter changes.
6) While waiting notice if there are any obvious air leakage sounds in any part of the system or vessel, if any area is suspected, then a spray of water in the affected area would reveal leak if there is bubbling present.

7) After waiting 10 minutes open the air supply valve again and note if rotameter is indicating any air flow in the system. System is hermetically sealed if there is no extra air flow in the system after opening it after 10 minute test.

If there is a leak it should be noted and leak should be addressed by replacing the offending part or replacing the seal.

30. Help

For user convenience a copy of the user manual, which describes all relevant procedures and principles of working with the BioReactor system is provided within the bioprocess controller. To access the mentioned manual press the Help button, which is displayed on each window of the system (see Figure 30.1).

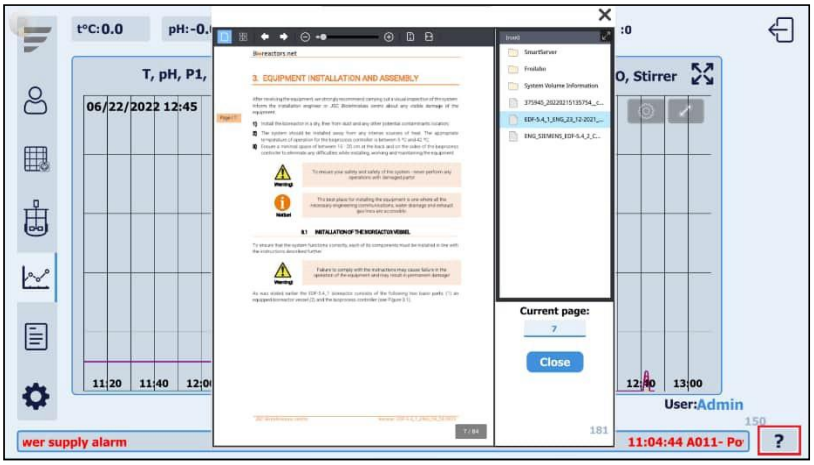

Figure 30.1. Help button

[END]