Standardized manual

KeBPr-1141 BIOSTAT® Cplus (5L)

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Writer: Tero Eerikäinen

1 Intended use

BIOSTAT Cplus bioreactor is meant for laboratory-scale (max 5 L) microbial cultivations / fermentations. The reactor is in-situ sterilizable. Also an external steam generator is required for the sterilization of the bottom and sample valves. The digital control unit (DCU) of the reactor has a touch-screen. This instruction includes only limited information of some basic operation. To build the system one has to find out more detail instructions from “Operating Manual BIOSTAT Cplus” which exists in pdf and printed format.

2 Safety

When sterilizing the reactor or the valves, don’t touch the metal parts without gloves, because they might be extremely hot and there is a risk of burns. Also face visor is recommended for protecting from steam and steam leaks. ALWAYS PUT ON the metal shield to cover the glass of the reactor which will protect from explosion in the case of the glass breaking. During the sterilization the inner pressure of the reactor rises and there is the risk of the glass breaking, resulting in a burst of hot liquid, steam and pieces of glass.

3 Instructions

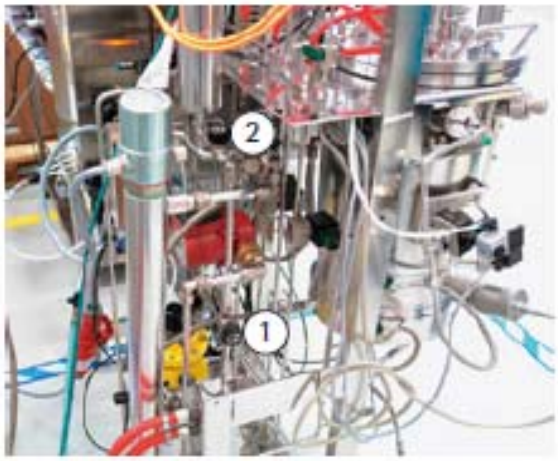
3.1 Before the cultivation with Biostat Cplus:

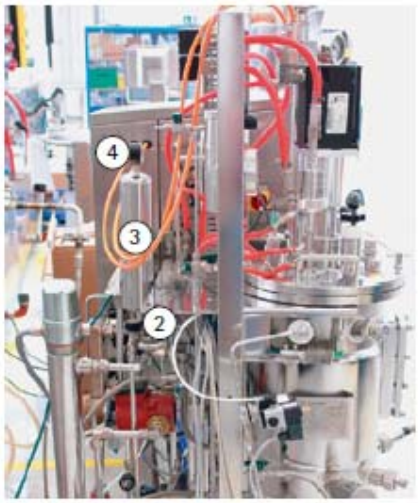
Autoclave the next / needed parts:

- Acid/base/antifoam bottles and their tubings / lines if needed
- Inoculation bottle and its line
- Possible sample plugs (e.g. for the Jipster line)
- Possible sample needle (e.g. for Jipster)
- Feed line
- Overflow pipe / outlet for the chemostat
- Feed bottle with antifoam and magnetic mixer inside
- Waste bottle

Open valves for water and air. Running the system dry can cause damages to the thermostatic pump! Do not switch on the bioreactor until the temperature control circulation has been filled.
For initial startup of the bioreactor or after refitting and/or maintenance work, the temperature control circulation must be filled with water.

Prior to filling, check that all fittings are tightened firmly. After filling, check for any visible leaks. If there are, do not start up operation of the temperature control system, but eliminate the cause of the leak.





Filling the temperature control circulation

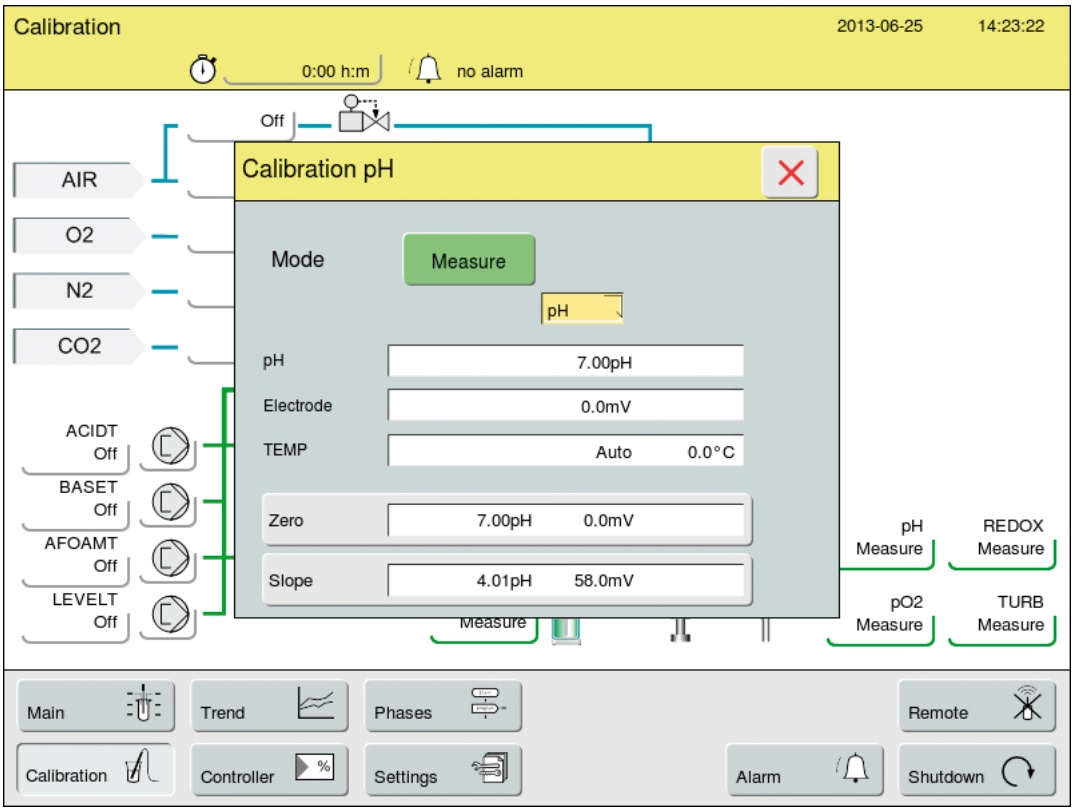
1) Open the ball cock “cooling water supply” (1) and ball cock “cooling water
2) overflow” (2).
3) Add water until filling sounds are no longer audible. Water should come out of the laboratory drain without bubbles. Close the ball cock “cooling water overflow” (2).
4) The temperature control circulation has the pressure of the cooling water supply (3 bar (4)).
5) Switch on the control unit (red switch on the side). Check the temperature setpoint. To prevent unnecessary heating at this time, set a setpoint in the range of ambient temperature (about 20 ° C). [Setup information for temperature control can be found in Manual Part B: Digital Measurement and Control Unit].
6) Let the thermostatic pump run for some time. Observe the pressure gage (4) on the expansion tank (3). If a pressure drop occurs, bubble noises are audible or bubbles appear in the water outlet, repeat steps 1 and 2.
7) Close the ball cock “cooling water supply” (1).
8) Open the ball cock “cooling water overflow” (2) again a little and observe the pressure gage (3). Once it shows a pressure of 0.5 bar (g), close the ball cock cooling water overflow (2) (*In the original manual here is a mistake!*).

The thermostat cycle is ready for operation. During sterilization the pressure of the water circulation usually rises a little bit over 2 bars.

3.2 Probe calibrations

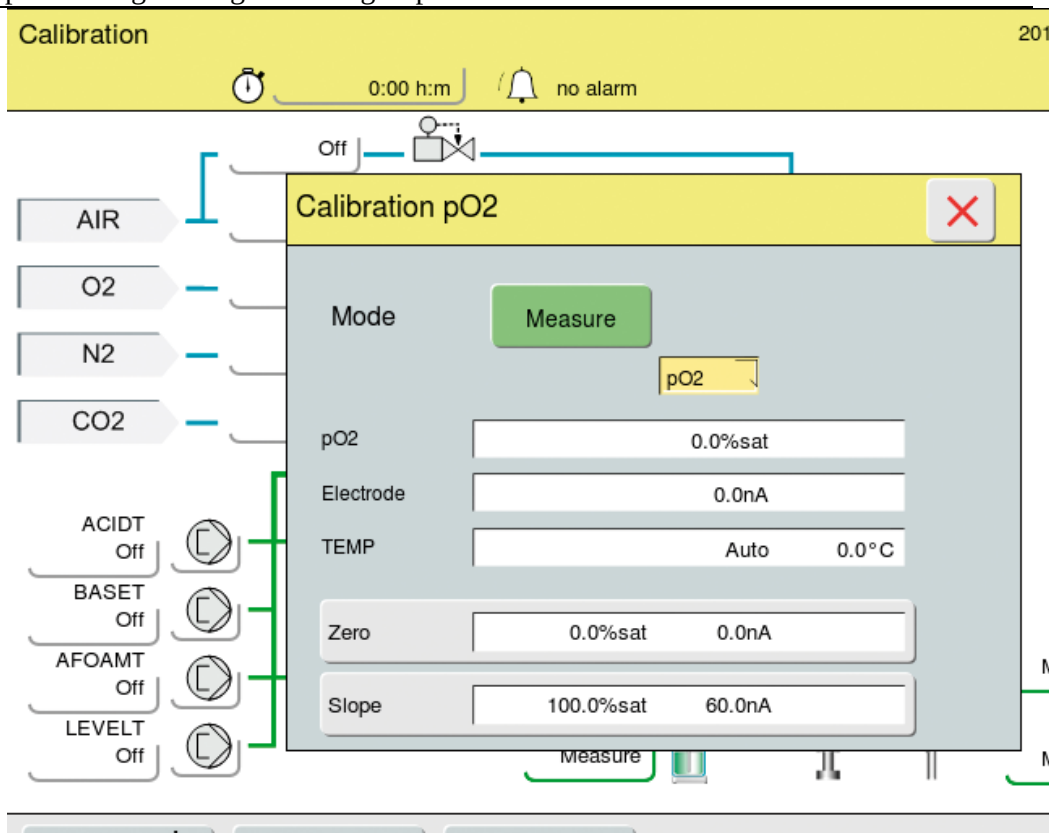
Connect a pH-probe to DCU and calibrate it with proper solutions. Choose the calibration mode from the DCU’s screen by pushing the “Calibration”-icon from the down list, the choose “pH”. For the calibration temperature, choose “auto” since the

calibration solutions are usually in room temperature (the same as the vessel, unless the reactor has been used very recently). If solutions are in some other temperature, then choose another temperature and add it manually. Choose “Calibrate” and follow the instructions on the screen.



The dissolved oxygen (pO2) probe is usually good to flush with water before attaching it to the reactor (you can find instructions for the calibration of the DO sensor and its parameters in manual “Part B: Digital Measurement and Control System”). Every now and then it’s good to open the probe, check / clean the membrane and add O2-electrolyte inside. The membrane should be intact and clean, if not, it should be changed. First option is to calibrate pO_2 sensor separately before sterilization by follows:

1) Check the condition of the pO_2 sensor. Open the membrane cartridge and check that there is electrolyte liquid inside (about 1.5 ml Oxolyte when filled). Turn on the equipment and connect the sensor to the control unit. Select a pO_2 sensor calibration. After a period of 2 hours or longer the sensor is ready for calibration. Such a polarization time is necessary to get stable signals. The following rule is valid if the sensor is disconnected from the amplifier for a short time:
   Polarization time = 2 x time of disconnecting, but no more than 2 hours. When stabilized the Electrode-value should be a stable value in the range of 40-80 nA:



2) Mark the Electrode-value of pO_2 sensors in the log book. If the value is not within 40-80 nA range, the sensor requires cleaning or electrolyte or membrane replacement.
3) Calibration includes the setting of the electrode zero point and the measurement of slope.
4) For installing in the vessel, insert the DO sensor into a 25 mm side port d 25 mm. Carefully tighten the screw connection finger-tight. Be careful also when pushing the probe inside so that you don't open the probe thread. If it opens then the electrolyte flows out and you can't calibrate the probe and you have to start from the beginning emptying vessel.
5) Calibrating the zero point: Aerate the culture medium using nitrogen until the dissolved oxygen is fully eliminated.
6) Calibrating slope: Aerate the medium with air or gas mixture.

If needed calibrate tubidity sensor and the necessary pumps (acid, alkali, and antifoam) (see Chapter 16 in BIOSTAT_Cplus-manual").

3.3 Sterilization

- Check whether all components and accessory parts have been installed that you will need for the process and that must be installed before sterilization.
- The pH sensor must be calibrated. If necessary, do this before filling the vessel.
- Close the floor drain valve and any ports and accesses that are still open.
- Close the sampling valve and | or the built-in intake valve.

- Make sure that exhaust gas -outline is directed to waste bottle / can and **NOT** to mass spectrometer or gas/CO2-analyzers.
- **Install the splinter protection sheath in 5 L culture vessels** [see in the manual Section *6.12.2 Splinter Protection Setup for 5-liter Culture Vessels*].

Sterilization of the culture vessel takes place in multiple steps in a defined sequence and is started and stopped from the operator terminal. The following assemblies are sterilized together with the culture vessel:

- Aeration segment
- Exhaust air segment
- Built-in assemblies such as sensors, agitator, etc.
- Floor drain valve (on vessel)
- Sampling valve (on vessel)

The following components, if present, must be sterilized manually after sterilization of the culture vessel:

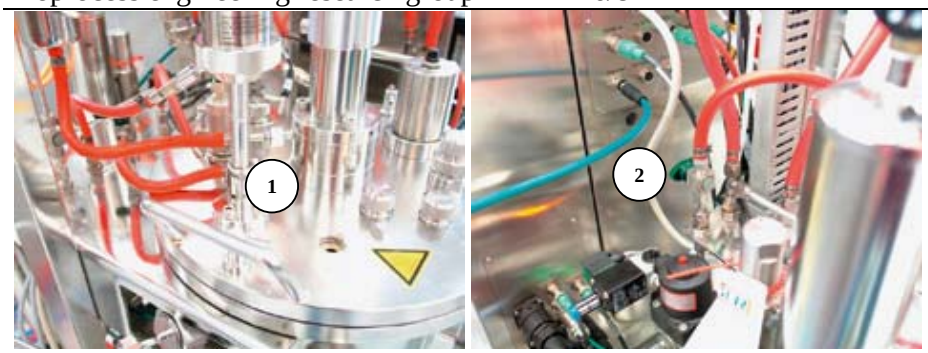
- Floor drain valve (exhaust side)
- Sampling valve (sampling side)

To compensate for the vacuum in the vessel after sterilization, the culture vessel must be aerated with 0.5 vvm (based on the max. working volume of the culture vessel) at a preliminary pressure of 1.5 bar (g). Setup must take place at the beginning of vessel sterilization.

Fill the culture vessel with at least 50% of the maximum operating volume. Note that liquid will vaporize during sterilization. The loss due to vaporization can only be determined by observation. If your system also has the empty sterilization function, steam (and thus condensate) is also conducted into the culture vessel during full sterilization. The resulting fluid loss/gain can only be determined empirically.

Attach all of the hoses to their right positions and the cords of probes to the side panel (left side) of DCU. If you are using the overflow pipe (always in chemostates) you need to autoclave it and attach it to reactor before sterilization. The holes of reactor's lid are closed with septum on top of which one puts a neck part and a plug. Also "high level" -probe (the thin black cord) needs to be unattached of the DCU during sterilization. In this 5-liter fermentor there is no double mechanical seal and hence no need for separate sterilization of the seal. Ensure that all culture vessel openings are closed and attachments are correctly and firmly attached.

- On the air rotameters (sparger & overlay), set an overall gas flow of 0.5 vvm based on the max. operating volume of the culture vessel.
- For gas paths with an installed mass flow controller, completely open the precision adjustment valve on the rotameter.
- Lift the supply air filter adapter "sparger" (1) into the "sterilization" position.
- Close the ball cock on the cooling water supply to the exhaust cooler (2).
- Start the full sterilization sequence at the control system.



The sterilization-menu is chosen from the main menu by pushing the “Phases”-icon in the down list, then choosing “S-FVES”. Then the right sterilization conditions are chosen: “Stemp” is the sterilization temperature (usually 121 ºC) and “Ftemp” fermentation temperature (the temperature to which the medium is cooled down after the sterilization is finished), respectively. “Stime” is the sterilization time (usually 20 minutes). Start the sterilization phase by pressing the “State” key and selecting the “start” mode.

3.4 Option: DO-probe calibration after sterilization

pO2-probe can also be zero-calibrated during the sterilization, after the temperature has reached over 100 ºC, when there shouldn’t be anymore dissolved oxygen left in the medium. The electrode is calibrated by choosing the calibration mode from the DCU’s screen by pushing the “Calibration”-icon from the down list, then choosing “pO2”. For the calibration temperature, choose “auto” since the probe is in the medium inside the vessel. Choose “Zero calibration” and follow the instructions on the screen. “Slope calibration” is made in a similar way, after the reactor has cooled down and the conditions are right and stabilized (usually just before the inoculation).

3.5 Sample valve sterilization during fermentation

Bottom and sample valves need to be sterilized separately with an external steam generator which is next to the reactor (check the standardized manual KeBPr-5237 for using the steam generator). You can sterilize them during the sterilization of the vessel or before starting the cultivation. The sample valve also needs to be sterilized after every manual sampling. Remember that after the steaming the valves are very hot which needs to be taken into account if taking samples. After the pressure of the steam generator is approximately 4 bars, the steam valve on top of the generator can be opened. Let the condensate first out of the fermentor’s valves by opening the small green valves below the reactor on the left side. Then close the valves and put on the steaming “plugs”. Open the green valves again and steam the valve(s) for 10 minutes. Close the green valves and let the “plugs” be attached to the valves, close the steam valve on top of the generator and close the steam generator. (”Blow” the steam generator if necessary).

When the automated sterilization program has reached to its end (i.e. temperature has cooled down to fermentation temperature), a red “Alarm”-light is flashing on the right side of the screen and there is a window on the screen saying “Sterilization is finished”. You acknowledge the alarm by pushing “Alarm”-button on the right and then “Ack” or “Ack all messages”. Open the refluxor (it’s good to open it immediately after the temperature is below 100 ºC). Move the aeration to “Ferm”-mode if cultivation is aerobic, so that the air goes below the liquid level. Attach the “High level”-cord if level

control is needed. Also attach the exhaust gas outline to mass spectrometer or CO_2-analyzer if you are using them.

3.6 Starting 'MFCS' programs / measurement

The main menu of 'MFCS'-program (MFCS-shell) is usually readily open, when the computer is on. If not, you can open MFCS-shell from computer's Start-menu → MFCS → MFCS-shell. After you have inoculated /started your cultivation, to start data-collection from the fermentation, push "Operator service" -icon from MFCS-shell's main menu. The on-going cultivations, their names and lengths are shown in the same window for each reactor. Push the "Start batch" -button. Name your cultivation and add additional information (e.g. inoculation time) if needed and push ok. Now MFCS is collecting data. If you want to see a graph of the different variables (e.g. pH, pO2, Temperature etc.) during a cultivation, push the "Plotting"-icon on MFCS-shell menu → choose your cultivation from the list, push right mouse-button and choose "Batch open" → choose the desired variables from the list with "Add->" → push OK. Now the MFCS draws a graph with the chosen variables from your cultivation. You can refresh the graph by pushing the red "!" (exclamation mark) -icon on the upper list of the graph-window.

3.7 Inoculation

Usually the inoculum is cultivated in Erlenmeyer-flasks in incubator with shaker. Before inoculation, the filtered medium components (if there are any) are added to the reactor through a septum on top of the reactor. Temperature, pH, airflow (gasflow) and stirring are put to "Auto"-mode. When the cultivation pH has been reached, acid and base totalizers are reset with "Start totalizer" -button inside acid and base menus (the list on the left on the main menu). Before inoculation, when the dissolved oxygen level is assumed to be on its maximum with the used stirring and airflow, the pO2-probe is slope-calibrated. The electrode is calibrated by choosing the calibration mode from the DCU's screen by pushing the "Calibration"-icon from the down list, then choosing "pO2". For the calibration temperature, choose "vessel temperature". Choose "Slope calibration" and follow the instructions on the screen. The reactor is inoculated through a septum on top of the reactor (syringe/needle or inoculation bottle with inoculation line). Immediately after inoculation, the data collection with MFCS is put on. Also measurements with gas mass spectrometer and/or CO_2-analyzer are put on if needed. Zero sample should also be taken right after inoculation (if needed). The timer on DCU's left upper corner in main menu should be reset at the time of inoculation.

3.8 For continuous operations

Medium components are dissolved in distilled water and feed is prepared as ready medium in a balance-tared bottle. The feed medium is then filtered with a pump through a sterilized 0,2 μm membrane filter cartridge to a sterile feed bottle, where there is antifoam and magnetic stirrer already in. In the cap of the feed bottle there are separate lines for feed inlet and feed outlet. Feed rate can be set/adjusted with a balance that is connected to DCU from the "Flow"-section (flow-unit is g/h) in DCU's main menu (the list on the left). The feed pump has to be on "Auto"-mode. When the feed is started, the waste bottle needs to be connected to DCU's "Level"-pump and to the hose of over flow pipe. The valve of the over flow pipe needs to be opened and "Level"-pump put to "Manual"-mode, so that it continually pumps the setpoint exceeding volume out of the reactor and keeps the volume in reactor constant.

3.9 On a daily basis

Manual samples from the reactor should be taken daily. After taking samples the sampling valve needs to be sterilized with steam. The samples can be analyzed for e.g. OD, dry cell weight and viability could be analyzed from the samples.

3.10 To end a cultivation

Data-collection with MFCS is stopped by pushing the “End batch” -button for the used reactor in MFCS-shell’s “Operator service” -window. Also CO_2-analyzer measurements is closed if it was used. If the cultivation medium needs to be killed (e.g. GMO-strain or hazardous/pathogenic strain), it can be done directly in the reactor just by starting the sterilization program (make sure that all the lines and holes in the reactor are closed and conduits removed and follow the sterilization instructions above). The medium can also be killed separately in an autoclave. If the medium needs to be post-processed or -analyzed, the medium is taken out of the reactor, filled with water and then sterilized (if sterilization is required). The reactor/vessel is cleaned with water (+ mild soap if needed) and brush. Every now and then it’s good practice to check the condition and cleanliness of the aeration filters (preferably after each cultivation).

4 Problem situations

In a problem situation where the problem can’t be solved with this manual, contact Tero Eerikäinen or Sartorius company’s Janne Mäkinen.