Standard Operating Procedure **SOP078**

Title: USE AND MAINTENANCE OF THE 5L BIOSTAT Bplus BIOREACTOR

Location: CBE Laboratory Unit, Laboratories H27/H29

1. PURPOSE

To describe the procedures for the use and maintenance of the BIOSTAT Bplus bioreactor and associated control systems and software in the CBE Laboratory Unit, specifically laboratories H25 (Animal Cell Culture) and H27/H29 (Microbiology).

2. SCOPE

This SOP describes the routine procedures, associated hazards and risk mitigation involved in the operation and maintenance of the BIOSTAT Bplus 5L bioreactor.

The BIOSTAT Bplus is a benchtop bioreactor system suitable for the culture of microbial and mammalian cells with the basic unit comprising of a 5L culture vessel with storage bottle tray, stainless steel housing, digital controller, operating interface and four peristaltic pumps. The culture vessel is equipped with sensors for monitoring pH, pO_2, foam and liquid level, stirrer shaft, three impellers, aeration tube with sparger, and a sample/harvest pipe.

3. RESPONSIBILITES

The Operator:

a. Must ensure familiarity with the equipment controls and requirements by reference to this SOP and the Operator and Maintenance Manuals, including operating instructions, record keeping and emergency shutdown procedures.

b. Must ensure that they have the proper authorisation to use the bioreactor, by completion of the "BIOSTAT Bplus 5L Bioreactor (FSOP078.2) Recording the appropriate information, instruction, and training in their training file.

c. Must ensure that after use the bioreactor is in a condition suitable for use by the next user ie the bioreactor is 'made safe' by running a decontamination autoclave cycle to deactivate any biological agents and by manually cleaning the vessel to ensure it remains in good working order and fill in the maintenance log sheet (FSOP078.4)

d. Must complete the checklist before each operational fermentation run so that all components are checked off when disassembling the unit to ensure any missing parts are identified as early as possible (FSOP078.1) If missing parts are identified this should be reported to the Responsible Person who will order replacements

e. Must assess their own capability with respect to handling the vessel. If a user is not comfortable when lifting the vessel from the bench to the trolley before transporting to/from room H31 for autoclaving the vessel must not be moved until a laboratory user capable of lifting the bioreactor

is available to help. Two people are always required when moving the trolley, but due to spatial limitations, a lab user must initially lift the bioreactor vessel alone.

f. Must complete the appropriate risk assessment before beginning any work with the BIOSTAT Bplus.

g. Must not tamper with BIOSTAT Bplus or its components (especially the PC). The PC is an integral part of the system – it must not be used as a general PC for the lab.

h. Must not make any changes to the BIOSTAT Bplus or its components as this can invalidate the warranty. Contact Sartorius for changes.

The Responsible Person/Laboratory Manager:

a) Organise maintenance, servicing and repair work. This may only be performed by fully trained, authorised personnel. The bioreactor must have been autoclaved and cleaned and a Decontamination Certificate (QS-FORM-009) must have been filled out prior to the arrival of the engineer who will perform the maintenance/servicing/repair work.

b) Record all maintenance, servicing and repair work should be recorded and filed with the BIOSTAT Bplus equipment file.

c) Ordering new parts for the bioreactor when identified by the operator

d) Ensure that all operators are authorised to use the bioreactor and that all authorised users have been given appropriate information, instruction, training and supervision in the correct use and maintenance of the BIOSTAT Bplus to ensure they and others are adequately protected.

4. EQUIPMENT AND MATERIALS

The following equipment and materials will be required for the use and maintenance of the Biostat Bplus bioreactor:

- Biostat Bplus Bioreactor (Model number: 8834314)
- Air filters
- Computer with MFCS/DA software
  Pumped liquids:
  - Polypropylene bottles
  - Media
  - Acid
  - Alkali
  - Antifoam
- Cleaning:
  - 1% Virkon solution
  - 70% IMS

- Calibration Liquid (pH values are at room temperature):
  - Buffer at pH 4.01
  - Buffer at pH 7.00
  - Buffer at pH 9.21
- Consumables:
  - Tubing A (4.8mm)
  - Tubing B (6.4mm)
- Transportation:
  - Trolley

NOTE: Consult relevant COSHH forms for any liquids used with the bioreactor.

5. PROCEDURE

5.1: Assembling the culture vessel

1. Ensure all equipment parts are dry before reassembling by wiping with tissue paper where necessary.

NOTE: There are times during vessel assembly where two people may be required.

2. As the vessel is assembled, fill out the checklist provided (see Section 8). This will be used to refer back to after an operational fermentation run to ensure that any missing components are identified early so immediate action can be taken to avoid disruption of future fermentation runs.

5.1.1 Connecting the ports

(i) Ensure that all the port connectors are attached to their correct port - Table 1 and Figure 1 below indicate the standard configuration for the vessel head plate. **NOTE:** Probes should not put in place at this point. Tighten all hexagonal nuts using a wrench (14mm diameter).

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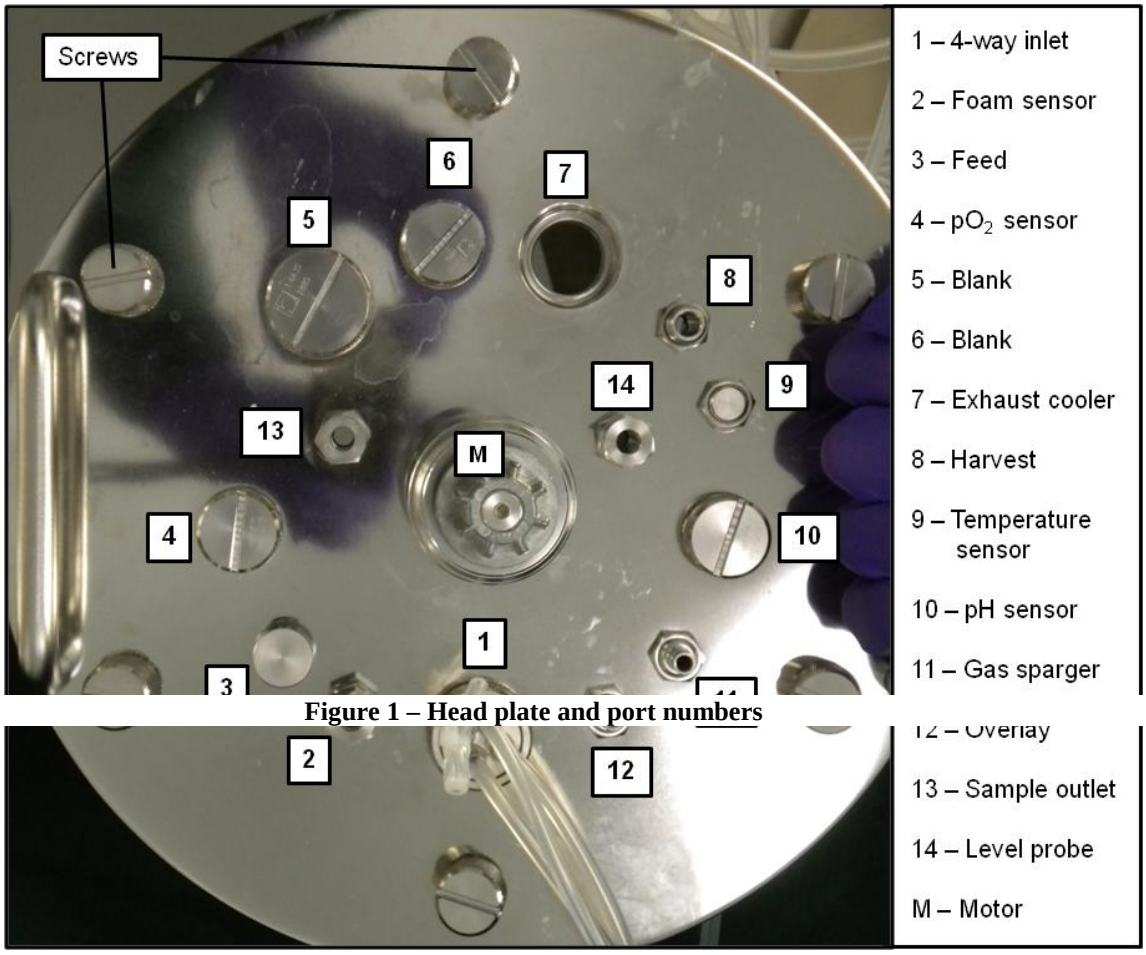

Figure 1 – Head plate and port numbers

Table 1 – Table listing the components of each port

Port Number	Port name	O-ring	Nut	Hexagonal adapter & plastic component	Stopper	Other components
1	4-way inlet	1	-	-	-	Large, removable screw-ring
2	Foam sensor	2	1	1	-	
3	Feed	1	1	1	-	
4	pO_2 sensor	-	-	-	-	
5	Blank	1	-	-	No.2885	
6	Blank	-	-	-	No.2810	Rubber cover for stopper (instead of O-ring)
7	Exhaust cooler	-	-	-	-	
8	Harvest	1	1	-	No.2782	
9	Temperature sensor	2	1	1	-	
10	pH sensor	-	-	-	-	
11	Gas sparger	1	1	-	-	
12	Overlay	1	1	-	-	
13	Sample outlet	2	1	1	-	
14	Level probe	2	1	1	-	
		16	8	5	3 (1)	

(ii) Impellers are attached to the motor drive shaft by sliding them along the shaft to the desired impeller height (predetermined by the user for each individual process). Use an Allen key (width: 3/16in or ~4.76mm) to tighten the impeller in place.

NOTE: Different types of impeller are available. It is important the user determines a suitable impeller number, height and type before assembling the vessel for sterilisation.

(i) Place the stand over the glass vessel so that the top jacket connector is between the open section of the stand. When operating, ensure the water jacket connectors face away from the operator for ease of operation.

NOTE: One person should lift the glass vessel slightly and hold it while the second person screws the supports back in place using an Allen key (width: 3/16in or ~4.76mm), so that the vessel rim rests on the frame.

(ii) Return the head plate to the glass vessel and screw back in place. Ensure that it is aligned so that the exhaust cooler port (Port 7) is be behind the motor, for ease of operation (see Figure 1).

(iii) Carefully push the antifoam, temperature and level probes into their respective ports (2, 9 & 14 respectively – see Figure 1).

NOTE: Slightly loosening the hexagonal adapters eases insertion (see Figure 2). The insertion height of the antifoam sensor is fixed whereas the insertion height of the level sensor depends on the intended culture medium filling level. This is measured later in Section 5.1.4.

NOTE: Labelled filling volumes of the culture vessels indicates only (roughly) estimated values. The exact correlation of filling volumes and medium levels must be measured with respect to the internal equipment of the vessel.

(vi) Once the probe is in place, retighten the hexagonal adapters by hand. Ensure the probes are as dry as possible around the connector and not screwed in too tight (particularly the temperature probe).

(v) The temperature probe-cable should remain attached to the probe (it is simply disconnected from the control tower). It should be shortened with a cable-tie to ensure that the connector does not fall into the water when autoclaving.

NOTE: Moisture can distort the measured temperature signal. When sealing the sensors tightly a vacuum can form during autoclaving. This can allow condensate to enter the connector and cable via the sensor which will damage the probe and cause inaccuracies in the temperature readings.

NOTE: The pO_2 and pH probes are not attached to the head plate at this point. They are both susceptible to drying if left in air and the pH electrode is calibrated outside the vessel before reattachment to the head plate. See sections 5.2.1 & 5.2.5 for probe reattachment.

(iv) Carefully push the pipes for the sample, feed and harvest ports in ports 3, 8 & 13.

(v) Assemble the sample kit by placing the 3-way connecter through the plastic lid (the side with the 3 short openings goes through the hole – see Figure 3 for a diagram of the sample tube). The O-ring is then placed around the 2 pipe-openings (on the other side of the connector) and placed so that it sits inside the lid. A small piece (~2cm) of the larger diameter (”B”) tubing is attached to the longest pipe. The glass tube can then be screwed in place around the 2 pipe-openings.

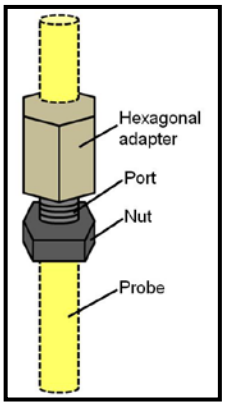

Figure 2 - Components of a smaller probe-port. (Port no's 2, 3, 8, 9 &13)

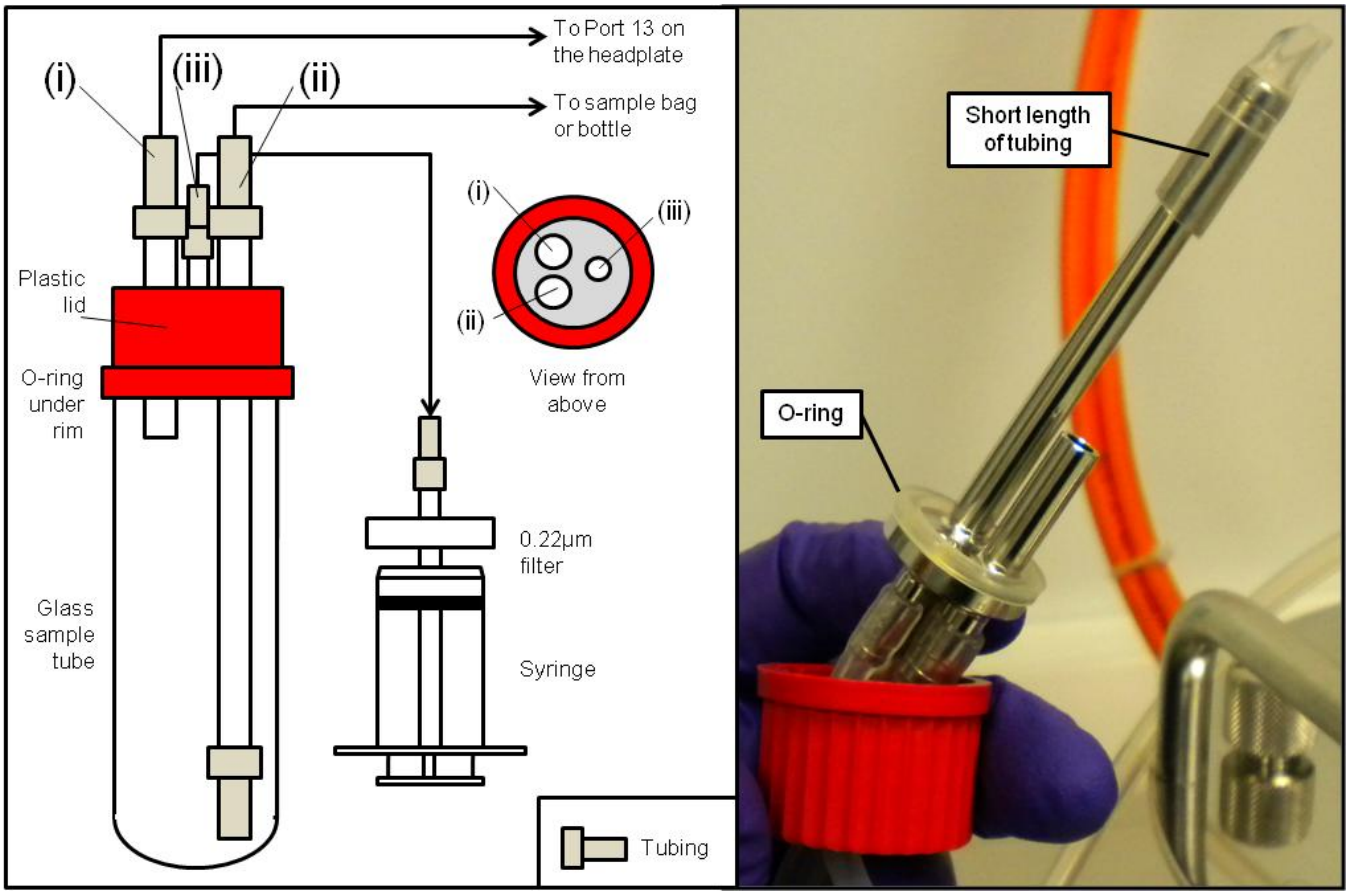

Figure 3 - Diagram and image illustrating the set-up of the sample tube

5.1.2 Pump calibration

Before attaching tubing to the head plate, the pumps MUST be calibrated:

NOTE: For the calibration of a pump, always use the same tubing as you would for the delivery of media during the process.

(i) To gauge the delivery, place one end of the tubing into a beaker filled with water and the other end into a 250mL measuring cylinder.

(ii) Ensure that the tubing is completely filled with water prior to starting the calibration so as to compensate for the clearance volume. Enable the pump manually until the tubing is filled up to its end.

(iii) Using "ACID" as an example, select the "Calibration" button from the main functions along the bottom line.

(iv) Select "ACID" from the "Totalizer" menu. The "ACID Totalizer Calibration" display will appear and. Select the "Mode" option and press "Start Calib.". Water will then be pumped into the measuring cylinder.

(v) Stop the pumping of the water after a period of time by pressing the "Stop Calib." option.

(vi) Determine the volume of water pumped into the measuring cylinder and enter this value into the "Quantum" menu. The system will then calculate the specific delivery by taking into account the running time of the pump and the gauged volume and displays the value in the "Flow" option.

(vii) If the specific delivery of the pump is known, this can be directly entered into the "Flow" menu.

(viii) Following pump calibration the user must fill out the “BIOSTAT Bplus Bioreactor Testing and Examination Log” (FSOP078.3). In the case of a fail, record in the Maintenance and Service Log (FSOP078.4) and contact the responsible person and/or the lab manager.

5.1.3 Tubing and air filters

New tubing and air filters should be put in place. Table 2 displays the amounts of tubing required.

Table 2 - Tubing and air filter requirements for each port

Port number	Port name	A Tubing	B Tubing	Air filter
1	4-way inlet	4	-	-
2	Foam sensor	-	-	-
3	Feed	-	1	-
4	pO_2 sensor	-	-	-
5	Blank	-	-	-
6	Blank	-	-	-
7	Exhaust cooler	-	1	1
8	Harvest	-	1	-
9	Temperature sensor	-	-	-
10	pH sensor	-	-	-
11	Gas sparger	-	2	1*
12	Overlay	-	2	1*
13	Sample outlet	1	1	-
14	Level probe	-	-	-
	Total:	5	9	1 (2*)

(Tubing A is the lower diameter tubing (4.8mm), and Tubing B has the higher diameter (6.4mm). * indicates that 2-way gas inlet filters are used rather than the ordinary disposable ones)

(i) Port 1 – 3 lengths of tubing A (~150cm each) are connected to the 4-way inlet with clamps placed near to the open end of the tubing. These will be used to feed acid, base or antifoam, or to adjust the level during the process, and so will be attached to bottles.

NOTE: A very short in length of A tubing (~5cm) will be required to seal the fourth opening of the adapter. This should be cable-tied to ensure no air can enter the system

(ii) Port 3 – A length of B tubing (~150cm) is connected to the port with the end sealed using a clamp.

(iii) Port 7 – The exhaust cooler is screwed into place and a length of B tubing (~40cm) attached to the opening on the top surface of the cooler. A clamp is used to seal this tubing.

(iv) Port 8 – Same as Port 3.

(v) Port 11 – See Figure 4. A short length of B tubing (~10cm) is connected from the port to opening (i). Another length (~20cm) is attached to the opening on the sloped side of the filter, with the end of the line clamped.

(vi) Port 12 – Same as Port 11.

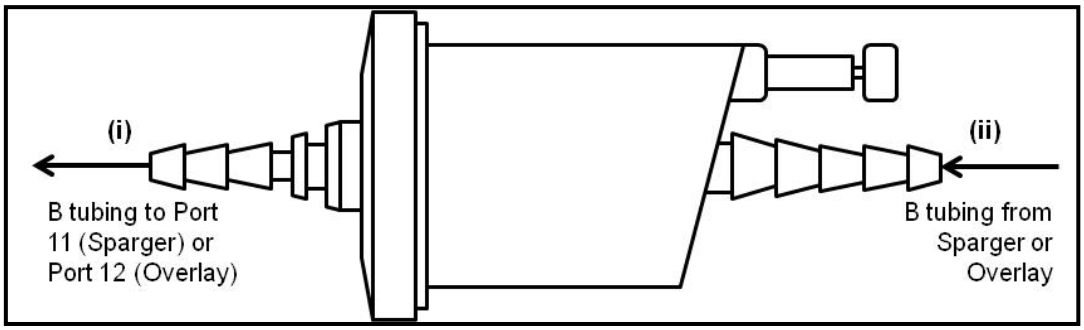

Figure 4 - Inlet gas filter for ports 11 & 12

(vii) Port 13 – See Figure 3. A length of B tubing (~30cm) is connected from port 13 to the opening (i) on the glass sample tube. Another, longer length of B tubing (~50cm) is connected to opening (ii) with the end clamped. A 0.20µm filter is placed on the end of a new syringe and a short length (~15cm) of A tubing connected from the end of this filter to opening (iii). The glass tube, syringe and associated tubing can all be placed on top of the bioreactor vessel when autoclaving.

5.1.4 Evaluation of insertion height of the level probe

The insertion height of the antifoam sensor is fixed. The insertion height of the level sensor depends on the intended filling level of the vessel with culture medium.

(i) Measure the filling level resulting for your intended medium volume, i.e. with water, after all intended equipment is installed in the vessel. Label the vessel accordingly.

(ii) Locate the level sensor at the height corresponding to the filling volume which must not be exceeded. Take into account that the level of the culture medium will rise a little at intensive mixing (high stirring speeds) and gassing.

(iii) For adjustment of the immersion depth of the level probe, the filling level obtained when using the intended working volume should be measured. The level should be labelled correspondingly on the vessel.

5.2 Preparation of the culture vessel for sterilisation autoclaving

5.2.1 Filling the water jacket

CAUTION: Possible damage of the vessel jacket at inadmissible overpressure. If the inlet and outlet connectors are mixed-up (e.g. if the male connector is attached at the lower nozzle of the vessel and the female coupling is attached at the upper nozzle) the jacket may be pressurised with overpressure of the cooling water supply and can burst. The user must be careful to ensure the jacket inlet and outlets are connected correctly as indicated on the side of the BIOSTAT control tower.

CAUTION: The upper connector MUST not be closed or clamped. The liquid in the jacket expands during heating up in the autoclave. Excess liquid must be able to flow out. After autoclaving the open connector provides compensation for vacuum of the jacket.

(i) Connect the vessel jacket to the BIOSTAT control tower. The inlet of the culture vessel is the lower connector with self-closing coupling; the female connector prevents draining of the jacket after disconnection of the tubing. The return line to the BIOSTAT control tower is the upper (open) male connector.

(ii) Press the large green button to switch on the control tower and enter the temperature control either from the main screen or from the controller screen. Press the “START” button to begin filling of the jacket.

NOTE: Ensure that no bubbles are visible in the jacket before starting any process run. Bubbles can easily be checked by examining the water inlet on the culture vessel.

5.2.2 Functional check and set-up of pO2 electrode

(i) Prior to each new run, the electrode should be checked to ensure the electrolyte solution (“OXYLITE”) is at the correct level. Every two months, the electrolyte should be changed using the method below with the change recorded in the maintenance file:

1. Remove the protective watering cap.
2. Unscrew the membrane cartridge and pre-rinse with OXYLITE solution. Wait until none is dripping down from the membrane (bottom of the electrode). Then fill the cap with 1.5mL of fresh OXYLITE.
3. Carefully screw the membrane cartridge so it is tightly in place ensuring the electrolyte doesn’t spill out.

NOTE: OXYLITE is corrosive. Always wear appropriate PPE as described in SOP 037 – Use of personal protective equipment (PPE). Small spillages should be flooded with water and mopped up with tissue paper.

(ii) Pre-autoclave functional check of the electrode:
The electrode MUST be polarized for at least 2 hours before calibration by simply connecting it to the BIOSTAT control tower with the system switched on. If the electrode is

disconnected for a short time during polarization, it should be connected again for a period of twice the length of time it was disconnected (as long as this is less than 2 hours).

1. With the BIOSTAT control tower switched on, and the pO_2 sensor connected, hold the sensor in the air and wait for a stable reading. Note this value.
2. Place the electrode in a bag containing pure nitrogen gas (>99.98%), and wait for a stable reading for the pO_2. The measured zero current in the oxygen-free environment must be less than 1.5% of the current in the air after 5 minutes.
3. Following the functional check, the user must fill out the "BIOSTAT Bplus Bioreactor Testing and Examination Log" (Section 8.3). In the case of a fail, record in the Maintenance and Service Log (Section 8.4) and contact the responsible person and/or the lab manager.
4. The sensor should be placed in port 4 on the head plate and screwed in carefully hand-tight – Do not use a wrench or similar tool to further tighten the nut.

5.2.3 Reactivation of pH-electrodes

For the first use or after long-term storage of the pH-electrode in a dry state, the electrode will need to be reactivated. The glass membranes of the pH-electrodes may have built up a thin watery gel layer due to a reaction with water. This layer will affect the measuring quality of the probe (response time, slope, alkali mistake) and therefore should be reactivated in order to ensure the pH probe is in working order.

(i) For reactivation, place the pH-electrode into a beaker for approximately 10 minutes with 0.1-1M NaOH, then for approximately 10 minutes into 0.1-1M HCl followed by 15 minutes in the storage solution supplied by the manufacturer.

5.2.4 Functional check of pH electrode

pH-electrodes are subject to wear and tear, e.g. caused by heating during sterilisation, by chemical reactions of the medium with the diaphragm or the electrolyte or through fouling of cells, cell debris and proteins.

(i) A functional check is limited to checking the electrode's zero and slope and comparing the pH-value measured "online" with the pH-value measured in a fresh sample.
(ii) Signs for wear and tear include an observed slower response, a reduced slope or a zero drift. If the measured pH-value is different for the medium at intensive stirring and at stopped stirrer drive, this may indicate fouling of the diaphragm.
(iii) Following the functional check, the user must fill out the "BIOSTAT Bplus Bioreactor Testing and Examination Log" (FSOP078.3). In the case of a fail, record in the Maintenance and Service Log (FSOP078.4) and contact the responsible person and/or the lab manager.

NOTE: If the pH electrode is placed into the slope buffer from the zero buffer particular care should be taken to avoid drying the diaphragm with paper cloths as this can affect the slope calibration. The electrode should be washed only with distilled water.

NOTE: The lifetime of the pH electrode depends on the number of sterilisation cycles and on the operating conditions in the process.

NOTE: Heat effects and reactions with components of media may affect the measuring quality of the pH electrode.

5.2.5 Calibration of pH electrode

(i) The pH electrode is calibrated by a two-point calibration. The calibration is done before mounting of the pH electrode in the culture vessel. It determines the "zero drift" and "slope" by measuring the pH value of the corresponding buffer solutions.

NOTE: Risk of burns by the calibration buffers. Wear appropriate PPE (e.g. gloves), as described in SOP 037, Use of Personal Protective Equipment (PPE) when handling the buffers.

(ii) For calibration, the reference temperature (current temperature of the buffer while filled in the beaker) is measured and entered in the calibration menu manually.

NOTE: The pH measurement during the process is takes into account the temperature measured in the bioreactor.

NOTE: The impact of heat during the sterilisation can cause a zero drift of the electrode. To compensate for such effects, the micro-DCU system allows for the user to recalibrate the pH electrode during the process. It is possible to measure the pH value in a sample taken from the bioreactor using a separate device and enter this value into the recalibration menu. The software recalculates the zero drift and displays the corrected "in-situ" pH value.

(iii) The calibration display of the pH electrode shows the calculated pH value, the measured electrode voltage and electrode parameters: "zero drift" and "slope". By monitoring these parameters, the functioning of the pH electrode can be easily checked.

i. From the main screen, press the "Calibration" button.
ii. Once the calibration menu has appeared, press the "pH" button, located in the "Sensors" menu.
iii. Press the "TCOMP" button and then select the "manually" option.
iv. Fill the zero buffer pH 7.0 in a beaker
v. Fill buffer solution of pH 4.0 or pH 9.0 (depending on the intended measuring range during the process) into another beaker.
vi. Measure the buffer temperature using a digital thermometer
vii. Press the button "TEMP"
viii. Enter the measured temperature as a reference value for the calibration and confirm by pressing the button "ok".
ix. Place the pH electrode in the beaker containing the zero buffer – if only the slope is to be calibrated, place the pH electrode in the beaker with the slope buffer.
x. Press the "Mode" option to select the intended step of calibration

1. Calibration = complete routine
2. Calib. Slope = slope calibration only (If this option is selected, only the slope calibration is performed).
3. Recalib zero = recalibration

xi. The zero calibration is started by selecting the "Calibration" option. The status display in the functional element "Mode" switches over to "calib".
xii. The window "ZBuffer" appears. You can enter the exact value provided on the label of the buffer bottle for the current buffer temperature or confirm the zero pH.
xiii. The calibration step starts after pressing "ok". A clock symbol appears which indicates that calibration is in operation.
xiv. The calibration step takes approximately 1 min. To interrupt the calibration, press the "Mode" option and select "Stop Calibration". The window "SBuffer" then appears.
xv. Remove the pH electrode from the zero buffer, wash with distilled water and place in the slope buffer, either pH 4.0 or pH 9.0
xvi. Enter the pH value of the slope buffer at the current temperature in the window "SBuffer" according to the intended measurement range during the process.
xvii. The exact pH value of the buffer at the reference temperature is shown on the buffer bottle.
xviii. Press the "ok" button to begin calibration. The clock symbol appears indicating calibration is in progress.
xix. The calibration step takes approximately 1 min. To interrupt the calibration, press the "Mode" option and select "Stop Calibration".
xx. After completion of the calibration the menu "Calibration – pH Sensor" appears once again. Change TCOMP back to "automatic".
xxi. Close the menu by pressing "exit"

6. Insert the pH electrode into Port 10 of the culture vessel and screwed in place carefully hand-tight – Do not use a wrench or similar tool to further tighten the nut.

NOTE: The pH measurement can be recalibrated if it is deemed that the measuring characteristics of the electrode may have been affected by the heat impact of sterilisation or by chemical reactions of the electrolyte with the culture medium. This is done by taking a sample of the culture and measuring the pH externally:

NOTE: Use only a fresh sample. Ensure that the pH value of the culture sample is not affected by the sampling process, the beaker or the storage until the pH is measured. To measure the pH of a sample, use a precise, carefully calibrated pH measuring device.

NOTE: If the currently measured pH value deviates largely from the pH value measured in the sample, verify the measuring of the sample before changing the calibration parameters. Input of incorrect parameters for recalibration affects the pH control – this can lead to an incorrect dosage of corrective solutions and affect the overall process.

I. Take a sample from the process and fill into a measuring beaker.
II. Measure the current pH value of the sample using calibrated pH measuring equipment.

III. Under the menu “Calibration” -> “pH”, select “Mode” and then select “Recalib.”.
IV. Enter the pH value measured in the sample.

V. The system then recalculates the zero drift and displays the corrected pH value.

VI. The calibration considers the following references and limits:

VII. Measured value of the electrode stable within 0.2% or
VIII. Measuring range for 60 sec.

NOTE: If the limits are exceeded, the system releases the error message “**ALARM** Calibr. Out of range”. The system still uses the zero found out and the slope for measuring of the pH. However, the measured pH values should be verified.

5.2.6 Filling of the culture vessel for autoclaving.

NOTE: Sterilisation of the culture vessel in a dry state will not ensure a complete sterilisation.

(i) The culture vessel can be sterilised with either culture medium or a small volume of water, depending on whether the medium is autoclavable.

(ii) If using autoclavable culture medium, introduce it through the feed port (no. 3) using an external pump. This entails placing the bioreactor on a trolley and taking the tubing from port 3 inside a BSC. The medium is then pumped from the BSC into the bioreactor.

NOTE: Some culture media will evaporate during sterilisation. The exact loss can only be ascertained empirically. The loss of media maybe compensated by increasing the quantity of culture medium before sterilisation or by introducing additional sterile medium afterwards (i.e. prior to inoculation and start of the process).

(iii) If the culture medium required for the process is non-autoclavable, water can be used as a replacement. The volume to be added should correspond to the loss through evaporation; recommended volume is approximately 100-200 mL. This can simply be poured in through one of the blank ports, which should then be resealed.

5.2.7 Filling of the corrective agent, media and harvest bottles and “trap device” for autoclaving

NOTE: The corrective agent, media and harvest bottles should be autoclaved **before** the culture vessel otherwise it may hold up the process.

NOTE: The required volumes of feed/harvest, acid, base or antifoam will vary from process to process. The exact amount needed should be calculated in advance and bottles selected that are large enough to handle the volume.

(i) If they are autoclavable, acid, base and/or antifoam corrective liquids should be added to bottles in a BSC. If they are not autoclavable, empty bottles should be prepared for sterilization at this point. The bottle caps have two openings: an air filter should be attached to the larger opening using ~10cm of B tubing at this point and the other smaller opening sealed using foil and cotton wool.

(ii) The medium should be present in sterile containers. It may be necessary to transfer this medium to a new bottle if, say the bottle caps that connect the tubing for the bioreactor do not fit the pre-sterilised medium container. In such a case, an empty bottle of predetermined volume should be attached to an air filter through the larger opening, and the smaller opening sealed using foil and cotton wool.

NOTE: If the connector screw-caps will fit the original medium bottle, one should be attached to an air filter via a short length of B tubing and sealed in an autoclavable bag for sterilization. This can later be attached to the medium bottle in the BSC.

(iii) An empty bottle will be required for the harvest. Again, this should be of predetermined volume, and given an air filter with the smaller opening sealed using foil and cotton wool. The tubing and filter requirements are summarised in Table 3 below.

(iv) An additional bottle will be required to act as a “trap device” for the exhaust cooler. This will require a 2-way adapter screw-cap with a short piece of B-tubing and an air filter attached to the larger opening. The open end should be sealed with cotton wool and foil.

NOTE: If a gas analyser will be used, an additional piece of tubing must be attached to the free end of the air filter and clamped. This is attached to the analyser following autoclaving using ethanol inside a flow hood (using the practices applied in Section 7.4.4)

Table 3 - Tubing and air filter requirements for additional bottles

Port number	Port name	Bottles	B Tubing	Air filter
1	4-way inlet	3	3	3
3	Feed	1	1	1
7	Exhaust Cooler	1	1	1
8	Harvest	1	1	1
	Total:	6	6	6

5.2.8 Handling & inspection of the culture vessels

CAUTION: Remove all cables before attempting to move the vessel for autoclaving. The cables MUST not be sterilised. The temperature probe is an exception to this. The cable cannot be removed from the probe and thus must be cable-tied to reduce its length, and will be autoclaved with the vessel.

NOTE: Handle the culture vessel with care during transport to the autoclave. Before placing into the autoclave ensure that no tubing or equipment mounted in the head plate can slip off unintentionally. Use autoclave tape to hold any loose tubing or equipment in place if necessary.

(i) Check that the ports to be utilised for the process are setup as necessary in accordance to the standardised head plate arrangement (Figures 2 & 3) where applicable. Ensure that remaining open ports are sealed off using the blank screw closures. All head plate screws should be tightened by hand.

(ii) Check the gas inlet tubing, the tubing of the corrective solution supplies and of all pipes which reach into the medium has been sealed by clamping. During autoclaving no medium must enter the tubing. Ensure that only the exhaust tubing is unclamped.

(iii) The stirrer drive motor is non-autoclavable; ensure it is not attached to the culture vessel.

(iv) In order to protect the electrodes from the impact of steam, cover the exposed plugs of the pH, pO_2, level and antifoam probes and cable connector of the temperature probe with aluminium foil.

(v) Before attempting to move the bioreactor a final inspection should be made to ensure no cracks are visible in the glass.

NOTE: There are 2 glass surfaces to inspect (the internal and external jacket walls.

(vi) A trolley must be used to transport the vessel to the autoclave room (H31). One lab user lifts the vessel onto the trolley and pushes/pulls it to the autoclave room, while a second person is present to ensure the vessel remains stable on the trolley.

NOTE: The vessel must be gripped from the bottom and held close to the body to ensure as little strain as possible is placed on the user. Manual handling training is recommended.

(vii) The loading support attached to autoclave 45 (in H31) must be used to place the vessel inside the autoclave. The operator should NEVER attempt to move the vessel between the lab and the autoclave room (H31) alone.

5.3 Autoclaving the culture vessel

NOTE: Danger of probes drying out – If using water to sterilise, ensure that there is enough time to autoclave the vessel, cool and fill with culture media so that the probes are not allowed to dry.

CAUTION: Possible damage of the vessel at inadmissible overpressure. If overpressure occurs in the vessel during autoclaving, it can be damaged. DO NOT clamp-off the exhaust tubing - the exhaust filter provides sterile pressure compensation with the interior medium compartment while the vessel is heated up to sterilisation temperature or cooled down afterwards.

(i) Ensure that the volume of liquid in the culture vessel is less than or equal to 5L. A second container (blank) must be filled with distilled water to a maximum volume of 2L. If, when autoclaved, the bioreactor contains less than 2L of liquid (media or water), then the blank must contain an equal volume of distilled water. The secondary container must be placed in the basket along with the culture vessel in a safe and appropriate manner.

(ii) The loading, unloading and operation of the autoclave must be completed as described in SOP 025, Use and Maintenance of the Systec VX95 Autoclave NO. CBE045.

(iii) The temperature in the centre of the culture vessel must be maintained at 121^{o}C for at least 20 min to ensure sufficient sterilisation.

(iv) The following cycles should be used depending on whether the vessel is filled with water or media:

Table 4 - Sterilisation cycles

Fill	Cycle No.
Water (Small volume)	1 – “Solids, instruments”
Autoclavable media	6 – “Sterilisation and Disposal of Liquid Waste”

(v) As soon as the sterilisation cycle begins, place the culture medium in a 37^{o}C incubator so that it will be warm when introduced to the vessel in section 7.4.2.

CAUTION: Fully loaded tubs/baskets/vessels may be too heavy to lift safely. To assist with the loading and unloading of the autoclave, an electrically-operated lifting device is available

(max 35kg). It is mounted directly onto the appliance and enables you to load and unload heavy loads without any exertion. The operation is aided by the use of a swivel arm.

(vi) Following the sterilisation cycle, if water has been used (rather than autoclavable medium) the vessel will need to be removed so it can cool as quickly as possible.

NOTE: If autoclavable medium was used, it will take significantly longer for the vessel too cool.

5.4 Cooling, filling and reconnection in preparation for a fermentation cycle

NOTE: Sections 5.4.1 & 5.4.2 can be skipped if the autoclavable media was used during sterilisation.

5.4.1 Cooling the vessel

CAUTION: Sudden changes in temperature can cause the glass vessel or jacket to crack. Ensure the vessel temperature has lowered significantly, and that the BIOSTAT control tower has been left switched on for a reasonable period with the temperature setpoint at 37^{o}C so that the temperature difference is not significant.

(i) After the 15 minutes has passed the vessel should have cooled significantly. Place it on the benchtop and connect the water jacket tubing and temperature probe cable to the control tower. Check the water that will enter the vessel jacket is at 37^{o}C before by entering the controllers menu and selecting “JTEMP”. The number below “JTEMP” on the resulting screen will indicate the temperature of the water inside the control tower.

(ii) Begin a jacket fill cycle and repeat until the setpoint is reached. (Refer to Section 5.2.1 for more information on filling the water jacket.)

5.4.2 Filling with culture medium

(i) Once the internal vessel temperature reads 37^{o}C (visible on the “MAIN” screen under the controller: “TEMP”) you may begin introducing the pre-warmed medium into the vessel.

(ii) The water jacket and temperature probe will need to be disconnected from the control tower and the vessel again placed on the trolley.

(iii) The tubing is connected to an external pump.

(iv) The open end of the feed line tube should be brought inside a BSC while the vessel remains on the trolley. The open end and outsides of the tube are soaked in IMS before the clamp is removed.

(v) The open end of the feed line is placed inside the bottle containing the medium. The pump is switched on and medium fed into the vessel.

(vi) Once the vessel has been filled, the tubing should be removed from the vessel and washed thoroughly with IMS before being sealed again with the clamp.

5.4.3 Reconnection of probes

(i) After autoclaving/before starting bioreactor operation, connect the Antifoam, Level, pH, pO_2 and Temperature probe cables to their respective sockets at their ports or on the control tower.

CAUTION: Never push the probe deeper into the vessel after sterilisation. This may introduce micro-organisms and cause infection. The O-ring inside the adapter seals the probe shaft against the adapter. It is possible to pull out the sensor a little even after sterilisation for readjustment, if inserted too deep.

5.4.4 Connecting external bottles, tubing and the motor prior to fermentation run

(i) Attach the bottle rack to the bioreactor stand.

(ii) Assuming the Acid/Base/Antifoam volumes were not autoclaved, these should be added to the now sterile bottles autoclaved previously (see Section 5.2.7). This must take place in a BSC.

(iii) The lids of these bottles should have 2 connectors for tubing – remove the foil and cotton wool from the openings. Place these lids onto the bottles for feeding media and collecting the harvest.

(iv) Disconnect the water jacket connectors and place the vessel onto the trolley and transport it over to the BSC – this will require 2 people to ensure the vessel is stable on the trolley.

CAUTION: Risk of dropping and breaking the vessel which may result in severe injuries from glass as well as acids/alkalis or reagents from the bioreactor. Lifting the vessel back onto the work surface requires it to be lifted above the height of the trolley. Ensure at least one additional person is present to ensure the extra containers do not slip from the trolley or to assist while the user places the vessel onto the lab surface. Do not attempt to lift the vessel alone. If the vessel is dropped then the appropriate action should be taken in accordance with SOP038 - Biological Spill Response.

(v) Use IMS to maintain sterility while connecting the tubing for the corrective fluid, “trap device” and media feed/harvest bottles (from Ports 1, 3, 7 & 8) – Outside the BSC, spray the opening of the tube before bringing into the BSC and removing the clamp. The tube can then be attached to the bottle opening.

NOTE: If a gas analyser is to be used, this MUST be connected via the open end of the filter from the “trap device”. This can be done outside the vessel.

(vi) Place the bottles in the corrective bottle rack (unless larger containers have been used) and place the extra containers for media on the trolley. Move all the containers and the vessel back to the BIOSTAT control tower. Again, 2 people are required. Reconnect the jacket vessel connectors.

(vii) Spray the open ends of the clamped tubing with IMS from the sparger and overlay ports (11 & 12) before attaching them to the openings on the BIOSTAT control tower. The openings are labelled on the tower (the upper connector is for the overlay).

(viii) Connect the red tubing from the exhaust cooler to the side of the BIOSTAT control tower. The connection points are just above the water jacket tubing point. The tower is also labelled to direct the user.

(ix) Attach the motor – Loosen the screw on the bottom rim of the motor and place over the connection point in the centre of the head plate. Tighten the screw.

5.5 Basic operation of the micro-DCU system

As mentioned previously in section 5.2.1, the BIOSTAT control tower is switched on by pressing the green button under the touch screen. This brings up the main screen. The basic functions are explained below:

5.5.1 Selecting Functional Groups and Submenus

The operating functions of the system are organised in functional groups, the "Main Functions":

(i) "**Main**": Visually displays the setup of the bioreactor and allows for the direct access to various submenus and functions required for operation.

(ii) "**Trend**": Allows for the visual monitoring of the parameters.

(iii) "**Calibration**": Allows the user to access submenus for operating the calibration routines of probes and totalizers.

(iv) "**Controllers**": Allows the user to access submenus for setting and adjusting the parameters of the various controllers.

(v) "**Maintenance**": Allows the user access submenus for general system settings, system reset,

(vi) "**Remote**": Allows for the switching over between local and remote operation.

(vii) "**Alarms**": Visual display of alarm messages in the event of an alarm.

(viii) **"Trend" Menu Display**: Allows for the monitoring and graphical display of various process parameters over a certain time period of the run. This provides the user with

process data which would enables the user to evaluate the run and identify the effect of certain events or control actions on the process.

NOTE: On the "Trend" display, the user can select to view up to 6 of 24 process parameters. The time period can be adjusted to allow for process to be viewed from 1, 12 or 72 hr.

NOTE: Trends are not stored. To record the run, store the data and view the process parameters for the duration of the run, the user must use the Multi-Fermenter Control System (MFCS/DA) software. The trend menu only allows the user to view the run for a maximum period of 72 hr and cannot be stored.

5.5.2 Selecting a new "Time Range":

NOTE: Selecting a new time range deletes the current record of data.

(i) Select the "Trend" button from the main functions along the bottom line.

(ii) In the headspace of the "Trend" menu, select "Settings" option. The "Settings" submenu appears.

(iii) Select the desired time range (1, 12 or 72 hr) by pressing the appropriate option.

(iv) Confirm by pressing "ok" or cancel and quit by pressing "C"

5.5.3 Adjusting which process parameters are selected

In order to view a particular process parameter:

(i) Select the "Trend" button from the main functions along the bottom line.

(ii) In the headspace of the "Trend" menu, select the unneeded process parameter. This brings up the "Channel Parameters" submenu.

(iii) Press the button "PV" on the submenu.

(iv) The submenu "Select Process Value" appears and lists the various process parameters which are available to be monitored. Use the arrow and slider to move down the list.

(v) Once located, select the desired process parameter and confirm selection by pressing "ok".

5.5.4 Changing the display range of a PV value:

(i) Select the "Trend" button from the main functions along the bottom line.

(ii) In the headspace of the "Trend" menu, select the unneeded process parameter. This brings up the "Channel Parameters" submenu.

(iii) Press the button "Range" on the submenu.

(iv) The submenu "Select Range" appears and lists the various configured ranges. Use the arrow and slider to move down the list.

(v) Once located, select the desired range and confirm selection by pressing "ok".

(vi) If the changes should be ignored, quit "Channel Parameters" with "C".

5.6 pO_2 probe calibration

Before the fermentation process can begin it is necessary to calibrate the pO_2 probe:

(ii) The “zero” and “slope” of the pO_2 electrode are calibrated in the culture vessel after autoclaving. The “zero” calibration is the electrode current reading when there is no oxygen in the medium and “slope” is the reading after saturation of the medium with the maximum supply of air or gas intended for the process.

(ii) The pO_2 electrode must be polarized for at least 1 hour before calibration by connecting it to the BIOSTAT control tower with the system switched on. If the electrode is disconnected for a short time during polarization, it should be connected again for a period twice the length of time it was disconnected (as long as this is less than 1 hour).

(ii) Before starting the "zero" calibration switch on the nitrogen supply and gas the medium until the oxygen dissolved in the culture medium is exhausted (dependent on culture volume).

(ii) To perform the calibration:

1. Open the "pO2 Sensor Calibration" screen by pressing the button "Calibration", and then selecting "pO2" from the "Sensors" list.
2. Select "TCOMP" followed by "manually" in the pop-up window
3. From the "pO2 Sensor Calibration" screen, select the "TEMP" button, and in the pop-up window enter "25^{o}C" and press "OK".

(ii) "zero" calibration:

1. Switch on the nitrogen supply at the tap (ensuring it is connected to the BIOSTAT control tower)
2. Open the Overlay valve fully by turning the knob counter-clockwise. Leave for 10 minutes to allow the nitrogen gas to displace any remaining oxygen in the medium or water contained in the vessel.
3. Enable the calibration by pressing the "Mode" button on the "pO2 Sensor Calibration" screen and select "Calib. Nitro".
4. Enter "0%" for "Nitrogen" and press "OK" to begin the calibration step. The clock symbol indicates the measurement is taking place. This should run for about 1 minute in total and will end automatically once the measured current stabilises. This step can be interrupted (if there is a stable measurement at near 0.0nA) by pressing "Mode" followed by "Stop Calib."
5. When the calibration is over, close the Overlay valve fully and shut off the nitrogen tap.

NOTE: "zero" calibration can also be performed by taking the pO_2 reading using whichever liquid was in the vessel during autoclaving. The heat from the autoclave should have degassed the liquid completely. Any remaining oxygen in the vessel would be picked up by the sensor.

6. At this point the system will automatically attempt to begin the "slope" calibration step. Cancel this by closing the "Air" menu by pressing "C" and quit the calibration by selecting "Exit". (If it the vessel was autoclaved containing culture medium, jump to part 8.
7. On the "Main" screen, press the "STIRR" button and use this function to incrementally increase the stirrer speed to the maximum rpm intended for use in the culture process.
8. Open the air valve fully and leave until the medium is saturated with oxygen. Saturation is indicated by a constant measured pO_2.

(ii) "Slope" calibration:

1. Return to the "pO2 Sensor Calibration" screen by pressing the button "Calibration", and then selecting "pO2" from the "Sensors" list.
2. Press the "Mode" button, select "Calib. Air" and enter "100%". Press "OK". As with the "zero" calibration, the clock symbol indicates the measurement is taking place. This should run for about 1 minute in total and will end automatically once the measured current stabilises. This step can be interrupted (if there is a stable measurement at near 0.0nA) by pressing "Mode" followed by "Stop Calib."
3. When the calibration is over, the mode display switches from "calib." to "meas.". Select the "TCOMP" button again and switch back to "automatic".
4. Close the Air valve fully and leave the calibration menu screen by pressing "Exit".

5.7 Recording the batch:

(i) Load the "Operator Services" MFCS/DA programme on the designated computer.

(ii) Ensure that the appropriate bioreactor control tower is enabled to allow for remote access from the computer. This is done by:

1. Selecting the "remote" control function from the main screen of the micro-DCU system
2. Select the "host" option
3. Press "enable".
4. The control tower is now connected to the PC which allows the user to control the bioreactor from the PC.

NOTE: When controlling the bioreactor from the PC, there can often be a delayed response when making a change or accessing certain features (approximately 3-5 secs). DO NOT attempt to repeat the command unless it has exceeded this time limit and is clear the input function was not received.

(iii) To select the correct bioreactor process unit on the MFCS/DA software, press:

a. "Select" from the menu bar at the top of the screen.
b. Then click on either "previous process unit" or "next process unit" options.

NOTE: To ensure that the readings of the fermentation will be recorded, a batch must be started. This doesn't necessarily mean the actual experiment has started, however this instructs the software to begin recording.

NOTE: The user has the option of starting the actual fermentation from the micro-DCU system or from the PC. Control can be established for up to 3 vessels using this system.

(iv) To begin a batch, ensure that the correct bioreactor has been selected and select the "start batch" option on the main screen.

(v) The recorded data may be viewed both during and after the experiment. To view the data select:

a. "Run" from the main screen
b. Select "Plotting"
c. This brings up a screen of the various batches, double click on the batch of interest
d. The "plot attributes" screen appears and the user can determine which parameters to view by selecting the parameter and pressing the "add" button".
e. To remove a parameter, select the "remove" button
f. Once the parameters have been selected and added, press "ok" and the software will prepare a plot of the specified parameters against time.

(vi) The data can be further manipulated by exporting the data to Excel. In order to do so:

a. Select the "plot setup" icon from the icon menu
b. Select "Export to Excel" option
c. Select "Run" and then "data export".
d. To select a particular batch, double click or right click on the appropriate batch which then exports the data to Excel.
e. The location of the file will be in the MFCS_DA folder, which depending on the setup of the computer should be: **C:\MFCS_DA\Database\Batches**.

5.8 Main Menu

The "Main" menu provides a graphical overview of the setup of the bioreactor and allows for direct access to important submenus and functions for operation. It comprises of 3 sections:

1 - The headline: This displays the status information of the system
2 - The working area: This displays the functional elements and submenus
3 - The bottom line: This displays the "main function" keys which allow for the user to switch between the "main function" menus.

Once a functional element or "main function" menu has been enabled, the background of the icon turns green. Below is a summary of the functions accessible from the main screen:

Table 5 - List of functional elements and their applications

Functional element	Displayed value	Submenu application
STIRR	Stirrer speed (rpm)	- Input of stirrer speed setpoint - Mode selection for stirrer controller
TEMP	Measure temperature of the culture vessel	- Input of temperature setpoint - Mode selection for TEMP controller
pH	Measure pH value	- Input of pH setpoint - Mode selection for pH controller
pO2	Measured value of the partial pressure of oxygen in the vessel [% pO2]	- Input of pO2 setpoint - Mode selection for the pO2 controller
FOAM	State of controller (on/off)	Mode selection for the FOAM controller
LEVEL	State of controller (on/off)	Mode selection for LEVEL controller
Valve	Output of valve [%]	Switch valve on or off
Air, O2_T, N2_T, CO_T2	Supply of air, oxygen, nitrogen or carbon dioxide [L/min]	Calibration of totalizer and resetting the process values
ACID, BASE, AFOAM, LEVEL	Dosing volumes of the pumps [mL]	Calibration of totalizer and resetting the process values
Pump	Output (state) of the pump [%]	- Display of state - Mode selection for the pump.
SUBAT, SUBBT	Dosing volumes of the pumps [mL]	Calibration of totalizer and resetting the process values

NOTE: All the controllers accessible from the main screen can also be accessed by selecting "Controller" from the row of options always visible at the bottom of the touchscreen, and then selecting desired controller (e.g. TEMP) from the list of options.

NOTE: The "WEIGH" button has no use to the system - balance platforms are required to monitor the weight of the vessel.

5.8.1 STIRR

This function is used to control the stirrer speed for the system.

1. Press "STIRR" button on the main screen.

2. Enter a desired stirrer speed (rpm) into the box; click "AUTO" and then press "OK". The impeller will then accelerate up to this speed quite quickly.

NOTE: Increasing the stirrer speed too rapidly can damage the motor. During processing, incrementally increase the stirrer speed by no more than 50 rpm at a time until the desired speed has been reached.

3. To stop the impeller, in the "STIRR" window, click "OFF" followed by "OK".

5.8.2 “TEMP” function and jacket fill

This function is used to control the temperature of the vessel by increasing or decreasing the temperature of the water entering the jacket.

1. Press the "TEMP" button on the main screen.
2. Enter a temperature value, click "AUTO" and then press "OK". The BIOSTAT control tower will then increase or decrease the temperature of the water entering the jacket until the inside of the vessel reaches the set temperature point. This will be maintained by the BIOSTAT control tower until the value is changed by the user.
3. To disable the temperature control click "OFF" followed by "OK".

Filling the jacket:

NOTE: Ensure the water is switched on at the tap before filling the jacket.

1. From the main screen, click "TEMP".
2. In the bar at the top of the window, click the => icon also labelled "TEMP" to go to the TEMP controller screen.
3. Press the "START" button on the right hand side of the screen. The jacket should be filled at least 3 times before each run and should be monitored visually. Whilst filling is in progress, a clock will appear to show that the system is running the operation.

NOTE: Ensure that no bubbles are visible in the jacket before starting any process run.

5.8.3 “pH” function

1. Press the "pH" button on the main screen.
2. Set the desired pH value to be maintained during the culture, click "AUTO" and then press "OK". The BIOSTAT control tower will then monitor the pH of the

culture using the pH probe, and automatically pump acid or base from the external containers to adjust the pH as necessary.

5.8.4 “pO2” function

1. Press the "pO2" button on the main screen.
2. Set the desired pO2 value to be maintained during the culture, click "AUTO" and then press "OK".

5.8.5 “FOAM” function

To configure the response of the FOAM sensor:

1. Press the "FOAM" button on the main screen.
2. Press the "=>" button that appears in the bar of the resulting window to go to the controller screen.
3. Press "SENSI" and reduce the sensitivity down from level 4 until foam is only just detected.
4. Press "CYCLE" to adjust the cycle time and "PULSE" to adjust the dosing time in accordance with the requirements of the process.

NOTE: If the foam control function is not required, the pump: "Antifoam" can be released for use as a substrate pump. To do this, press the button labelled "PUMP" and select "----".

To enable the FOAM sensor:

1. On the main screen, press the "FOAM" button.
2. Press "AUTO" and then "OK" to enable the system to pump antifoam into the system when required.

NOTE: The Pt-100 temperature sensor provides the reference potential for the antifoam sensor. Therefore the foam control can only function if both these sensors are installed in the culture vessel.

5.8.6 “LEVEL” function

The pump controlled by via the level probe is able to function either to feed media into the vessel (to a height controlled by the probe) or harvest the vessel contents to an external container.

To select and configure the desired mode of operation:

1. Select the "LEVEL" button on the main screen
2. Press the "==>" button to go to the controller screen.
3. Press the button labelled "PUMP" and change the pump type to either "Harv" (Harvest) or "Feed".

To enable the level sensor:

1. On the main screen, press the "FOAM" button.
2. Press "AUTO" and then "OK" to enable the system to pump antifoam into the system when required.

5.8.7 Pumps

The buttons for pumps: "ACID", "BASE", "AFOAM" and "LEVEL" listed on the main screen all jump directly to their respective "Totalizer Calibration" screen. See Section 5.1.2.

5.9 "Maintenance" Menu Display

NOTE: Unauthorised changes of settings in the "Maintenance" setting can potentially damage or cause the bioreactor to malfunction. The user only needs to enter this section to adjust the time/date on the bioreactor.

5.9.1 General System Settings:

The "Maintenance" menu display has a few options to choose from, however the user may only enter "System Parameter" menu if the internal clock on the bioreactor requires changing. The user must not enter any other menu.

5.9.2 Changing the time on the bioreactor

(i) Select the "Maintenance" button from the main functions along the bottom line.

(ii) Select the "System Parameter" icon. This displays the current time, date and failtime of the bioreactor.

NOTE: The user should only change the Time and Date, not the Failtime.

(iii) To change the time, select the "Time" option, this displays the "Enter Time" menu. Once the time has been entered, confirm by pressing "ok" or cancel by pressing "C"

(iv) To change the date, select the "Date" option, this displays the "Enter Date" menu. Once the date has been entered, confirm by pressing "ok" or cancel by pressing "C".

5.10 "Alarm" Menu Display

If an alarm has occurred during a process run, the "Alarm" menu function along the bottom line will change colour from green to red. The user can press the "Alarm" menu function to determine the reason for the alarm. The user should refer to the table below for the meaning of a particular alarm and what remedial action should be taken.

Table 6 - Alarm messages and responses

Alarm Message	Meaning	Remedial Action
Process Alarms		
"Name State alarm..."	Alarm of digital input "name"	Confirm with "ACK"
"Name low alarm..."	The process value "name" is below its lower limit threshold	Confirm with "ACK"
"Name high alarm..."	The process value "name" is below its upper limit threshold	Confirm with "ACK"
"Heater failure..."	Overheat protection of thermostat cycle (jacket filling) is activated	Jacket must be refilled with water
"Power failure..."	Mains failure has occurred	Display only; system restarts automatically when the mains supply is re-enabled.
"Motor failure..."	Overheat protection of motor is activated	Allow motor to cool down before switching on bioreactor again
"TEMP Sens. failure..."	Pt-100 in culture vessel is defective or cable not plugged in	Check cable/plug, if connection is ok, sensor maybe defective
"JTEMP Sens. failure..."	Pt-100 in thermostat cycle is defective	Requires replacing, inform Lab Manager
Process Messages		
"Shut down fermenter..."	Emergency cut off - key of	Switch on bioreactor by pressing

	bioreactor enabled	the SHUT DOWN key once again
"Name <3 mA alarm..." "(Name of an analogue input) <3 mA alarm"	The analogue 4.20 mA input has a broken cable	Check cable connection and connected peripheral unit
System Alarms		
"DFC Rest HH:MM..."	System confirms enabling of a system reset via the maintenance menu, with time of release ("HH" and "MM" minutes ago)	Confirm with "ACK"
"(Task name) Watchdog Reset"	Confirmation of watchdog reset, caused by malfunctioning of micro-DCU system; with source of malfunction	Make a note of alarm message and inform Lab Manager to contact Sartorius; confirm with "ACK"
"Hardware Reset"	Confirmation of a hardware reset, resulting from a system reset.	Make a note of alarm message and inform Lab Manager to contact Sartorius; confirm with "ACK"
"Power failure..."	Mains cut off occurred "HH" hours and "MM" minutes ago	Confirm with "ACK"
"Pwf stop ferm HH:MM..."	Mains failure occurred "HH" hours and "MM" minutes ago; max interval of mains failure passed.	Confirm with "ACK"
"Printer not ready"	Printer protocol enabled but data transmission to printer is impossible	Check connection to printer; switch over printer into "Online" mode

5.11 disconnecting the Culture Vessel

1. Upon completion of a run, the culture vessel will need to be decontaminated by autoclaving. However before proceeding, parts of the vessel must be disconnected first.

CAUTION: When disconnecting, the vessel must remain a closed system until after the decontamination autoclave cycle in order to protect the operators and the clean environment.

2. Only certain parts of the vessel should be disconnected and not the entire vessel itself, ensuring that its contents remain inside and do not spill out into the clean environment. The following tasks need to be completed:

(i) Disconnect the probe-cables from the control tower (ensuring the probes remain inside the vessel). The temperature probe-cable remains attached to the probe (it is simply disconnected from the control tower). It should be shortened with a cable-tie to ensure that the connector does not fall into the water during the autoclave cycle.

(ii) Ensure the water has been switched off before disconnecting the tubing for the water jacket.

(iii) Disconnect at the metal connection points in the jacket tubing. Please take care whilst uncoupling the connectors as some residual water in the tubing may spill out. In the event of such an occurrence, soak up with blue paper before wiping the spilled area using IMS.

(iv) Ensure any gases used have been switched off at the tap before removing the tubing.

(v) Clamp the tubes attached to external containers at appropriate length and disconnect all tubing from the bottles, the control tower and the sample tube (i.e. do not disconnect at the head plate).

(vi) Remove the corrective fluid bottle rack by loosening the screws at the attachment points on the stand; it can then be lifted up and away from the vessel.

5.12 Decontamination autoclaving

(i) If the liquid broth has been removed prior to completion of a bioreactor run, then a small amount of water should be added before autoclaving (~100mL). Cycle 1: “Solids, instruments” should be used to decontaminate.

(ii) If the broth is still present in the vessel, Cycle 6: “Sterilisation and Disposal of Liquid Waste” MUST be used for the decontamination run.

CAUTION: Possible damage of the vessel at inadmissible overpressure. If overpressure occurs in the vessel during autoclaving, it can be damaged. Do not clamp-off the exhaust tubing, the exhaust filter provides sterile pressure compensation with the interior medium compartment while the vessel is heated up to sterilisation temperature or cooled down afterwards.

NOTE: The centre of the culture vessel must maintain a temperature of 121^{o}C for at least 20 min to ensure sufficient sterilisation. This is verified by a probe in the “blank” water vessel.

(iii) The loading, unloading and operation of the autoclave must be completed as described in SOP 025, Use and Maintenance of the Systec VX95 Autoclave NO. CBE045.

(iv) Ensure that the volume of liquid in the culture vessel is less than or equal to 5L, and that a second container prepared with distilled water (blank) must equal the volume of the liquid in the culture vessel up to a maximum of 2L. The secondary container must be placed in the basket along with the culture vessel in a safe and appropriate manner.

CAUTION: Fully loaded tubs/baskets/vessels may be too heavy for safe manual lifting. To assist with the loading and unloading of the autoclave, an electrically-operated lifting device is available (max 35kg). It is mounted directly onto the appliance and enables you to load and unload the autoclave without any exertion. The operation is aided by the use of a swivel arm.

CAUTION: Risk of injury when handling heavy culture vessels. The culture vessel can be extremely heavy when filled with liquid and completely equipped, use appropriate lifting devices and ALWAYS wear appropriate PPE as described in SOP 037, Use of Personal Protective Equipment (PPE). Manual handling training is highly recommended.

CAUTION: Handle the culture vessel with special care while transporting to the autoclave. When placing into the autoclave take care that no tubing or equipment mounted in the head plate can slip off unintentionally.

5.13 Dismantling the culture vessel

(i) Disconnect remaining cables and tubing. Remove all electrodes and component parts, as far as necessary for cleaning and maintenance of the bioreactor.

(ii) Unscrew all head plate screws by hand from the flange only; they do not need to be removed fully from the head plate.

(iii) Life the head plate from the glass vessel – be careful when removing as the O-ring of the head plate can be strongly bound to the glass flange of the vessel.

(iv) Once the head plate has been removed, the glass vessel is removed from the metal stand. This is achieved by using an Allen key (width: 3/16in or ~4.76mm), to loosen the vessel supports.

CAUTION: The glass vessel may break if unsupported when removing the vessel from the metal stand. Two people will be required for this operation as when the supports are removed the vessel will drop slightly as it comes away from the metal stand.

(v) After the system has been completely disassembled, parts should be sorted and the checklist (FSOP078.1) from the previous run used to ensure none are missing. If any are missing or damaged, the user must fill in a Maintenance & Service Log (FSOP078.4). Refer to Table 1 for information on parts containing O-rings, nuts and probe adapters.

5.14 Manual cleaning of the vessel

After an autoclave decontamination cycle, it may be sufficient to simply flush the vessel carefully with water if there is limited time before the next process.

NOTE: During short breaks between processes, or if it is not possible to clean immediately, the vessel should be filled with water in order to protect the pH and pO_2 probes. If they are allowed to dry, they may be unable to function correctly.

During breaks between process runs, the reactor vessel MUST be disassembled and each part cleaned manually using 0.5M sodium hydroxide solution.

(i) All cables except that from the temperature probe should remain attached to the tower and only wiped with IMS to remove any noticeable dirt where necessary.

(ii) All parts are cleaned mechanically using 1% Virkon solution to remove any deposited material. The exhaust cooler, BIOSTAT control tower and the drive motor are special cases and are discussed later in of this section. Probes also have a separate cleaning protocol.

CAUTION: Virkon is corrosive and a strong irritant. Always wear appropriate PPE as described in SOP 037 - Use of personal protective equipment (PPE).

(iii) It is recommended that larger components such as the 5L glass culture vessel, stainless steel baffles, and the head plate should be scrubbed using a cloth and aquarium brushes used to scrub the ports, and smaller components such as the nuts and metal stoppers.

CAUTION: O-rings can be easily damaged when cleaning and so should be cleaned carefully using a cloth or blue paper rather than an aquarium brush. If O-rings are damaged, a report should be entered into the Maintenance & Service Log (FSOP078.4), Refer to Table 1 for information on parts containing O-rings.

(iv) Autoclavable bottles used to supply media or collect harvested material from the glass vessel should also be washed out by hand using 0.5M sodium hydroxide. Bottles containing an acid, base or antifoam should be rinsed with copious amounts of water before being washed with 0.5M sodium hydroxide and again rinsed with copious amounts of water before drying.

(v) When cleaning the exhaust cooler, check the O-rings and replace if dirty or damaged. Wipe externally using blue paper soaked in 0.5M sodium hydroxide if necessary to remove deposited material. If foam is thought to have entered the exhaust cooler, check the internal tubes which can be accessed from the bottom of the cooler (see Figure 5). Clean the internal tube if dirty using sodium hydroxide and an aquarium brush. Rinse thoroughly with water.

(vi) The outside of the BIOSTAT control tower should be wiped to remove any deposited material using blue paper soaked in 0.5M sodium hydroxide when necessary.

NOTE: Do not spray the touch screen with any liquid, and do not wipe except to remove deposited material or markings. Ensure liquid does not enter the inside of the BIOSTAT control tower along the edges of the screen.

(vii) Wipe the outside of the drive motor when necessary using blue paper soaked in 0.5M sodium hydroxide. DO NOT attempt to open up the motor.

(viii) All parts should be rinsed thoroughly with copious amounts of water before drying.

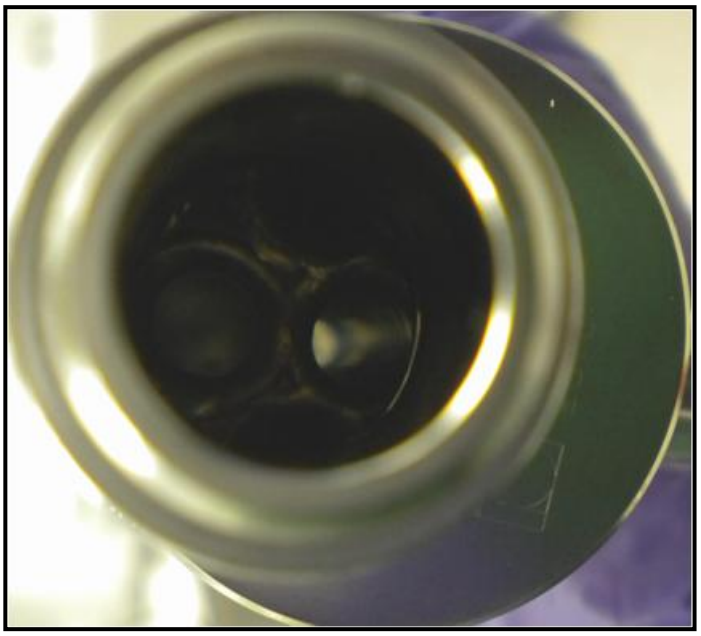

Figure 5 - Internal piping of the exhaust cooler

NOTE: The electrodes may become useless e.g. if they are fouled by cells, cell debris or ingredients of the culture medium and the layer dries.

(ix) pH and pO_2 electrodes:

1. The electrodes should be washed with distilled water after any run - Do not store dirty electrodes.
2. Check and clean the O-rings
3. For short-term storage, the sensors can be kept in demineralised water. For a longer storage you can place the pH-electrode in a beaker filled with 3m-KCL solution.
4. A small amount of the electrolyte can be filled into the protective cap delivered with the electrode and attached onto the electrode's tip.

(x) Once the cleaning of the vessel has been completed, the vessel can then be assembled again and sterilised before starting a new fermentation fun.

5.15: Equipment Malfunction

(i) If any part of the equipment fails or malfunctions, the user should contact the Responsible Person. With permission of the Responsible Person the user should consult the Operator Instruction Manuals to access fault finding and troubleshooting procedures.

(ii) All problems and corrective actions should be recorded in the Maintenance Log (FSOP078.4).

(iii) If the equipment fails to work or malfunctions and cannot be rectified according to troubleshooting procedures detailed in the Operator and Users Manuals the Responsible Person must be informed and a "Do Not Use" notice should be posted on the equipment. Contact the manufacturer for advice and coordinate with the Lab Manager for external maintenance and servicing.

(iv) External maintenance and servicing of the equipment can only be performed after it has been suitably disinfected (refer to SOP003 for further details) and a 'Decontamination Certificate' (QS-FORM-009) has been issued by the School/Building/Unit Safety Co-ordinator.

5.16: HEALTH & SAFETY

I. Hazard risk when transporting the vessel: The glass vessel of the bioreactor is quite heavy, particularly when filled with liquid. A trolley MUST be used to transport the vessel to/from the autoclave room (H31). The loading support attached to autoclave 45 (in H31) MUST be used to place the vessel inside the autoclave. The operator should NEVER attempt to move the vessel between the lab and the autoclave room (H31) alone.

II. Conduct a biological risk assessment before culturing any cells with the bioreactor and adhere to guidance at all times. If a genetically modified organism (GMO) is to be used in the bioreactor work, a Genetically Modified Organism Risk Assessment (GMO RA) must be completed before proceeding.

III. Always wear appropriate PPE when working with the bioreactor, this includes labcoat, gloves and safety spectacles (Refer to SOP037)

IV. Ensure that whilst in operation, the bioreactor is maintained as a closed system at all times. Loosening or removal of tubing compromises integrity.

V. NEVER attempt to dismantle the bioreactor once it is in operation or after the vessel has been autoclaved for sterilisation.

VI. Hazards associated with burns and scalds: The bioreactor vessel may be extremely hot following autoclaving. After autoclaving, the user should leave the vessel to cool down for 15 minutes before moving. During this period a warning notice should be placed beside the vessel, informing other lab users of the risk

VII. During operation, if the jacket temperature has been set to a high temperature, the user should not touch the glass vessel or metal head plate. If high temperatures are used, a notice should be placed beside the vessel.

8. DOCUMENTATION

Standard Operating Procedure **SOP078**

Title: USE AND MAINTENANCE OF THE 5L BIOSTAT Bplus BIOREACTOR

Location: CBE Laboratory Unit, Laboratories H27/H29

These forms are an output of this SOP and can be found on the CBE website:

FSOP078.1: Components Checklist

FSOP078.2: Training Agreement

FSOP078.3: Testing and Examination Log

FSOP078.4: Maintenance and Service Log

QS-FORM-009: General Decontamination Certificate

Standard Operating Procedure **SOP078**

Title: USE AND MAINTENANCE OF THE 5L BIOSTAT Bplus BIOREACTOR

Location: CBE Laboratory Unit, Laboratories H27/H29

SOP Version History

Version Reviewed	Date Revised/ Reviewed	Revision Summary	New Version Number
002	15.11.2012	Moved over to lean template. Removed sop history to the back, removed refernces, created FSOP forms. Written changes (K.Marrow/Q.Rafiq). New version needed.	003
003	18.6.2018	Change of location, from H25 to H29 Reviewed by (J.Ali)	004

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