



Table of Contents

1.0 Purpose	1
2.0 Scope	1
3.0 Authority and Responsibility	1
4.0 Materials and Equipment	1
5.0 Procedure Guidelines	2
6.0 Procedure	2
7.0 Documentation	14
8.0 References and Related Documents	14

1.0 Purpose

This procedure describes the operation procedures for the Sartorius BioStat STR Generation 3 50L Bioreactor.

2.0 Scope

This SOP applies to all Biopharmaceutical Development Program (BDP) personnel operating the BioStat STR. This bioreactor is a stainless-steel bag holder intended to house a single use vessel bag equipped with optical and/or conventional sensor ports. This single use vessel system will be used for mammalian cell culture production.

3.0 Authority and Responsibility

- 3.1 The Director, Technical Operations, BDP has the authority to define this procedure.
- 3.2 Production personnel are responsible for training on this procedure and for documenting training to Biopharmaceutical Quality Assurance (BQA).
- 3.3 Production personnel are responsible for the implementation of this procedure.
- 3.4 BQA is responsible for quality oversight of this procedure.

4.0 Materials and Equipment

- 4.1 BIOSTAT Flexsafe STR 50 L, BDP PN 22319.
- 4.2 O₂ Optical Sensor, BDP PN 22463
- 4.3 pH Optical Sensor, BDP PN 22464
- 4.4 pH 4 buffer, BDP PN 30107.
- 4.5 pH 7 buffer, BDP PN 30108.

- 4.6 pH 10 buffer, BDP PN 30109
- 4.7 70% Decon-ahol, BDP PN 30129 (or equivalent disinfectant)
- 4.8 WFI.
- 4.9 Nitrogen Gas (For DO probe testing).

5.0 Procedure Guidelines

- 5.1 Use the BPR and equipment logbook to document this procedure.

6.0 Procedure

6.1 Utility Startup

NOTE: Verify that all utility lines are hooked up to their corresponding supply/return piping before turning on any of the utilities to the vessel(s).

6.1.1 Electrical

- 6.1.1.1 Power on the main unit.
- 6.1.1.2 Power on unit for bag holder.

6.1.2 Compressed Air

- 6.1.2.1 Open compressed gas valve on the utility panel.
- 6.1.2.2 Verify that the supplied pressure is 2.0 barg (29 psi), maximum, as measured from the utility panel gauge.

6.1.3 Pharmaceutical Air

- 6.1.3.1 Open pharmaceutical gas valve on the utility panel.
- 6.1.3.2 Verify that the supplied pressure is 2.0 barg (29 psi), maximum, as measured from the utility panel gauge.

6.1.4 CO₂ gas

- 6.1.4.1 Open the CO₂ gas valve on the utility panel.
- 6.1.4.2 Verify that the supplied pressure is 2.0 barg (29 psi), maximum, as measured from the utility panel gauge.

6.1.5 Nitrogen Gas

- 6.1.5.1 Open the nitrogen gas valve on the utility panel.
- 6.1.5.2 Verify that the supplied pressure is 2.0 barg (29 psi), maximum, as measured from the utility panel gauge.

6.1.6 Oxygen Gas

- 6.1.6.1 Open the oxygen gas valve on the utility panel.
- 6.1.6.2 Verify that the pressure gauge reads 2.0 barg (29 psi), maximum, as measured from the utility panel.

6.2 Vessel Preparation

6.2.1 Flexsafe Bag Installation

NOTE: Flexsafe STR 50 L bag unpacking requires a minimum of two operators for safe installation. This will provide the appropriate structural support for all critical vessel components.

6.2.1.1 Place the Flexsafe STR 50 L (BDP PN 22319) bag shipping container on a flat, level surface. Cut the outer packaging away from the vessel bag shipping container while the container sits upright. Care should be taken to ensure that vessel bag or tubing lines are not damaged while cutting away any shipping components.

6.2.1.2 Remove the vessel tubes and drain lines from the lower foam shipping element. Remove the upper foam elements, and the four tube elements from the lower foam shipping element. Discard any removed shipping components.

NOTE: Ensure that the black fitting collar is saved and set aside before discarding any components.

6.2.1.3 To prepare the drain valve/fitting cone for installation, obtain the fitting cone collar and place the fingers of the right and left hand in the recessed grips provided. Pull the two fitting cone elements apart.

6.2.1.4 Lay the two elements around the drain port (it should be properly located below the drain port ring – push the elements together. An audible sound should be heard indicating the drain port retention ring is secure.

6.2.1.5 Remove the drain line tube from the dust bag. Position and close the clip as close as possible to the drain valve/fitting cone to reduce dead volume.

NOTE: Only lift and transport the bag using the hard, plastic components of the agitator connection and drain port.

6.2.1.6 Person 1 will lift the bag at the top agitation connection, Person 2 will lift/support the bag at the drain port.

6.2.1.7 Remove the bag from the lower foam shipping element and flip the bag so the top port is oriented upwards and drain port downwards.

6.2.1.8 Open the bag holder door. Lift the bag over the double outer wall into the bag holder.

6.2.1.9 Person 1 holds the top agitator connection which will connect to the magnetic coupling of the bag holder.

NOTE: The top port must be held in a vertical position until the magnetic coupling is connected to the top port.

- 6.2.1.10 Person 2 holds the drain port fitting cone with their right hand and guides the drain line through the drain port holder.
- 6.2.1.11 To close the drain port holder, press the handle in the direction of the drain port holder.
- 6.2.1.12 If the drain port holder cannot be close, check the position of the fitting cone, adjust if necessary and repeat the closing process.
- 6.2.1.13 To lock the drain port holder, turn the lever to the lock position.
- 6.2.1.14 Close and securely latch the bag holder door.
- 6.2.2 Setting up the Exhaust and Inlet Air Filters
 - 6.2.2.1 Remove the dust covers from the long tubes, e.g., feed tubes and connectors. Do not remove the dust covers from the short tubes, e.g., aeration tubes and exhaust tube. The caps of the short tubes can fall into the bag holder during setup.
 - 6.2.2.2 Remove the dust covers from the exhaust tubing and the exhaust air filter (BDP PN 22465) capsule from the packaging. Connect the exhaust air filter capsule to the exhaust tubing of the bag via the OPTA coupling.
 - 6.2.2.3 Adjust the setting height of the filter clamp on the filter holder so that the exhaust tubing is not kinked. Secure the filter holder clamp.
 - 6.2.2.4 Remove the dust cover from the inlet sparge tube. Remove the filter capsule (BDP PN 22466) from the packaging and connect the filter capsule to the inlet sparge segment via the OPTA coupling.
 - 6.2.2.4.1 Repeat the above step for the vessel overlay inlet tube and filter.
 - 6.2.2.5 Clamp the sparge and overlay filter capsules in position on the filter holder. Adjust the setting height of the filter clamp on the filter holder so that the aeration tube is not kinked. Secure the filter holder clamps.
 - 6.2.2.6 Connect the pressure sensor and the overlay tubing. Connect the pressure sensor and the control/supply unit with the overlay filter outlet port. The pressure sensor provides monitoring of the bag pressure and prevents over pressurization and failure of the sterile vessel.
- 6.2.3 Inflating the Vessel Bag
 - 6.2.3.1 Position the bag so that the sampling port and the sensor ports can be routed through the sensor window.

- 6.2.3.1.1 The bag must be connected to the drain port holder, the top port must be connected to the magnetic coupling of the bag holder as installed in step 6.2.1.
- 6.2.3.2 To inflate the Flexsafe STR 50 L bag (PN 22319)
 - 6.2.3.2.1 Adjust the setpoint for AIR-OVERLAY1 and AIR-SPARGER1 to 1.5 L/min. Verify the control loop on the screen in green.
 - 6.2.3.2.2 Aerate the bag without pressure by slowly increasing the aeration rate, from the previously established setpoint, until you reach the maximum of 5 L/min.
 - 6.2.3.2.3 When the bag has unfolded and is flat against the wall of the bag holder, stop aeration by turning off the control loop.
- 6.2.3.3 Once the vessel has completely inflated, monitor it for 5 minutes by checking for any visible leaks. Continue to the sensor/probe installation if the vessel bag does not exhibit any pressure leaks.
- 6.2.4 Installing the Vessel Temperature Sensor
 - 6.2.4.1 Open the screw of the temperature sensor fixture and insert the temperature sensor in the sensor fixture. Slide the sensor until the screw is above the insulation of the temperature sensor and tighten the screw.
 - 6.2.4.2 Insert the temperature sensor into the sensor port and secure on the tube nozzle of the port.
 - 6.2.4.3 Connect the signal cable of the temperature sensor to the M12 plug connection Temp of the control / supply unit.
- 6.2.5 Installing Single Use Optical pH/DO Sensor(s)
 - 6.2.5.1 Twist the knob on the single-use sensor port 90 degrees counterclockwise until it stops.

NOTE: When the knob is in the vertical position, the sensor port is enabled. The connector for the optical DO (pO₂) sensor is marked in red. The connector for the optical pH sensor is marked in white.
 - 6.2.5.2 Loosen the screw on the fiber optic cable fixture. Push the fiber optic cable out of the fiber optic cable fixture until the fiber optic cable protrudes 4-5 cm out of the fiber optic cable fixture.
 - 6.2.5.3 Tighten the screw on the fiber optic cable fixture.

- 6.2.5.4 To connect the optical DO (pO₂), press the fiber optic cable of the DO sensor in the connector of the single-use sensor port marked in red. Hold the knob and pull the spring to the single-use sensor port, slide the end piece of the fiber optic cable fixture all the way into the single-use sensor port. A clicking sound will indicate that the fiber optic cable is secured in the single-use sensor port.
- 6.2.5.5 To connect the optical pH sensor, press the fiber optic cable of the pH sensor in the connector of the single-use sensor port marked in white. Hold the knob and pull the spring to the single-use sensor port, slide the end piece of the fiber optic cable fixture all the way into the single-use sensor port. A clicking sound will indicate that the fiber optic cable is secured in the single-use sensor port.
- 6.2.5.6 Connect the fiber optic cables to the control / supply unit. Connect the fiber optic cable of optical DO sensor to the terminal DO-C1 opt. of the control / supply unit. Connect the fiber optic cable of the optical pH sensor to the terminal pH-C1 opt of the control / supply unit.
- 6.2.5.7 To calibrate the pH optical probe, navigate to the BioStat STR control tower display's main screen, then open the Calibration screen.
- 6.2.5.8 Select the button for the sensor to be calibrated (pH-#1)
- 6.2.5.9 This will prompt a submenu (Calibration pH-#1). Press the Inactive button, which brings up the Calibration Mode window. Press the (Enter init. Parameters) button. Enter the following parameters from the pH Calibration Data label, found on the vessel bag, one after the other in the respective window
 - 6.2.5.9.1 **Temp**
 - 6.2.5.9.2 **f (max)**
 - 6.2.5.9.3 **f (min)**
 - 6.2.5.9.4 **dpH**
 - 6.2.5.9.5 **dH0**
- 6.2.5.10 Confirm the parameters by pressing the OK button, then wait for the parameters to be transmitted. The window will indicate when it is complete.
- 6.2.5.11 At the same window, set the probe to initialize and press the Play button. This will initialize the optical probe using the input calibration which will take 2 hours to complete. The probe should not be used until initialization has completed.

NOTE: The vessel bag optical pH membrane has a 20,000 light pulse limit before significant drift will be observed. An appropriate pH measurement interval should be selected for the total run length in order to maintain accurate measurements for the complete process duration.

6.2.5.12 Repeat steps 6.2.5.7 through 6.2.5.11 for additional optical pH sensor calibrations.

6.2.5.13 To calibrate the DO optical probe, navigate to the BioStat STR control tower display's main screen, then open the calibration screen.

6.2.5.14 Select the button for the sensor to be calibrated (DO-#1)

6.2.5.15 This will prompt a submenu (Calibration DO-#1). Press the Inactive button, which brings up the Calibration Mode window. Press the (Enter init. Parameters) button. Enter the following parameters below from the DO Calibration label, found on the vessel bag, one after the other in the respective window.

6.2.5.15.1 **Temp**

6.2.5.15.2 **0 % sat**

6.2.5.15.3 **100 % sat**

6.2.5.16 Confirm the parameters by pressing the OK button, then wait for the parameters to be transmitted. The window will indicate when it is complete.

6.2.5.17 At the same window, set the probe to initialize and press the Play button. This will initialize the optical probe using the input calibration data which will take 2 hours. The probe should not be used until initialization has completed.

NOTE: The vessel bag optical DO membrane has a 200,000 light pulse limit before significant drift will be observed. An appropriate DO measurement interval should be selected for the total run length in order to maintain accurate measurements for the complete process duration.

6.2.5.18 Repeat steps 6.2.5.13 through 6.2.5.17 for additional DO sensor calibrations.

6.2.6 Installing Conventional pH/DO Sensor(s)

6.2.6.1 Consult the BPR or appropriate logbook for the serial numbers of product specific vessel probes required for use. A secondary pH/DO probe may be needed alongside the standard optical probes per the BPR.

- 6.2.6.2 To calibrate the conventional pH probe, navigate to the BioStat STR control tower display's main screen, then open the calibration screen.
- 6.2.6.3 Select the button for the sensor to be calibrated (pH-#1)
- 6.2.6.4 In the pH-#1 calibration fly-out list, select calibrate from the calibration drop-down list. Then select the play button which will prompt calibration.
- 6.2.6.5 The temperature compensation menu is displayed.
 - 6.2.6.5.1 To manually enter the temperature compensation, press the NO button, then enter the temperature compensation value
 - 6.2.6.5.2 To select the automatic temperature compensation, press the YES button
- 6.2.6.6 The input window pH-#1: Zero Buffer for zero-point calibration will appear
 - 6.2.6.6.1 Hold the pH sensor in the pH 7.00 buffer solution
 - 6.2.6.6.2 In the zero reference pH buffer field, input the pH value of the buffer solution.
- 6.2.6.7 The window for the pH-#1: Zero Value will appear
 - 6.2.6.7.1 Observe the measure value displayed
 - 6.2.6.7.2 Confirm the measurement by pressing the OK button as soon as the display is stable.
- 6.2.6.8 The window for pH-#1: Calibration Slope Buffer is displayed
- 6.2.6.9 Rinse the pH sensor with the second buffer and then hold the pH sensor in the second buffer solution
- 6.2.6.10 In the pH-#1: Slope reference pH buffer field, input the pH value of the second buffer solution.
- 6.2.6.11 The window for pH-#1: Slope Value will appear
 - 6.2.6.11.1 Observe the measured value displayed
 - 6.2.6.11.2 Confirm the measurement by pressing the OK button as soon as the display value is stable.
- 6.2.6.12 The pH sensor is calibrated.
- 6.2.6.13 Screw the sensor into the sensor adapter and insert the adapter into the autoclave tray.

- 6.2.6.14 Autoclave the autoclaving holder, the sensor adaptor and sensors in the autoclave. Use a sterilizing autoclave and the appropriate validated cycle for vessel probes.
- 6.2.6.15 After autoclaving the sensors, remove the autoclaving holder from the autoclave using the yellow handle. Hook the bracket of the autoclaving holder onto the upper system rail.
- 6.2.6.16 To calibrate the conventional DO probe, navigate to the DCU's main screen, then select the Calibration button to bring up the Calibration window.
- 6.2.6.17 Select the button for the sensor to be calibrated (DO-#1)
- 6.2.6.18 In the DO-#1 calibration fly-out list, select calibrate from the calibration drop-down list. Then select the play button which will prompt calibration.
- 6.2.6.19 The temperature compensation menu is displayed.
 - 6.2.6.19.1 To manually enter the temperature compensation, press the NO button, then enter the temperature compensation value
 - 6.2.6.19.2 To select the automatic temperature compensation, press the YES button
- 6.2.6.20 The input window DO-#1: Zero Buffer for zero-point calibration will appear.
 - 6.2.6.20.1 Mount the DO sensor on the bag.
 - 6.2.6.20.2 Fill the bag and condition to the desired temperature.
 - 6.2.6.20.3 Set the N2 aeration to 100%. Set all other aerations to 0%.
 - 6.2.6.20.4 Set the stirrer (STIRR) to approximately 80%-100% of the maximum speed.
 - 6.2.6.20.5 In the DO-#1: Zero Buffer, enter the DO value (0%)
- 6.2.6.21 The DO-#1: Zero Value window will appear.
 - 6.2.6.21.1 Wait until the oxygen dissolved in the medium has been displaced. When the raw electrode signal stabilizes near the 0 nA value, the oxygen saturation is approaching the minimum.
 - 6.2.6.21.2 Observe the measured value display in the DO-#1: Zero Value window. Once the display is stable, confirm the measurement by pressing the OK button.
- 6.2.6.22 Set the Air aeration to 100%. Set all other aerations to 0%.

- 6.2.6.23 Set the stirrer to approximately 80%-100% of the maximum speed.
- 6.2.6.24 In the DO-Slope Buffer window, enter the DO value (100%)
- 6.2.6.25 The DO-#1: Slope Value window will appear.
 - 6.2.6.25.1 Observe the measured value in the window. The raw electrode signal stabilizing near the maximum value of the DO sensor is an indication that the oxygen saturation is approaching the maximum
 - 6.2.6.25.2 Once the display is stable, confirm the measurement by pressing the OK button.
- 6.2.6.26 The DO sensor is calibrated.
- 6.2.6.27 Repeat steps 6.2.6.15 - 6.2.6.17 to sterilize calibrated DO sensor in autoclave.

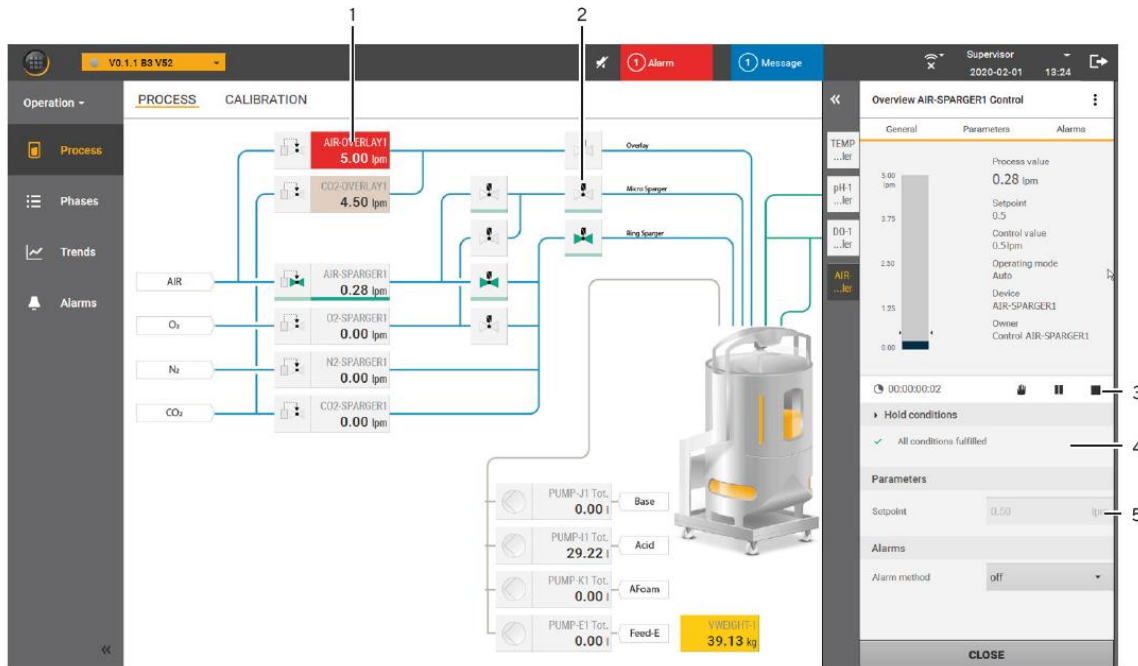
6.3 Vessel Operation

6.3.1 Vessel Media Addition

- 6.3.1.1 Verify that the STR 50 control tower is connected to the MFCS data capture software computer via the ethernet cable. Start a new batch within MFCS titled with the appropriate information from the project BPR.
- 6.3.1.2 Verify/close the vessel bag's bottom drain port.
- 6.3.1.3 Perform an aseptic addition, as directed in step 6.3.3., for as many times as required to reach the BPR specified starting volume.

6.3.2 Setting Vessel Control Loops

- 6.3.2.1 After adding the required amount of starting growth media, all process related control loops (i.e., Temperature, pH, DO) should be activated and placed into AUTO mode as specified by the BPR.
 - 6.3.2.1.1 To activate controller mode and setpoints, navigate to the STR control tower's main screen.
 - 6.3.2.1.2 Press the button for the loop to be changed (i.e., TEMP-#, pH-#1, DO-#1), which will bring up the Controller menu. The menu allows the user to change several different parameters including alarms and setpoint.
 - 6.3.2.1.3 The picture below shows an example of the Controller window for the air sparger parameter.



1. Control card of a control loop
2. Control card with integrated symbol
3. Phase control bar
4. Hold Conditions
5. Setpoint

6.3.2.1.4 To change the mode of the controller, press the Play button in the phase control bar, which will engage that controller's function. If selected successfully the button should change color depending on the operating mode chosen. Off will be gray, Auto will be green, Manual will be blue. Select the stop button in the phase control bar to shut off the controller's function.

6.3.2.1.5 To change the setpoint, press the Setpoint bar. A number pad will appear. Enter the desired setpoint, then press ok. The number pad will close.

6.3.2.2 Gas controllers control gas supply for the corresponding gas (ie. [AIR-OVERLAY1], [AIR-SPARGER1], [O2-SPARGER1], [N2-SPARGER1], [CO2-OVERLAY1], or [CO2-SPARGER1-] and introduce gas into the Overlay, Sparger, or Micro-Sparger (vessel bag configuration dependent.)

- 6.3.2.3 Enter flow rates and appropriate operating mode(s) as specified by the BPR by selecting each required gas controller at the main screen.
- 6.3.2.4 Select the BPR specified gas controller by pressing the corresponding name plate at the main screen.
- 6.3.2.5 Enter the specified setpoint and mode using step 6.3.2.1.3 as a reference for the interface. MIN and MAX output limits can be entered in the % of the control range of the gas feed if specified by the BPR. Press the close "X" button and repeat for all required process gases.
- 6.3.2.6 After enabling the appropriate control loops, from steps 6.3.2.1.1 - 6.3.2.5, verify that the vessel parameters equilibrate to all BPR operating setpoints. If the control loops do not equilibrate properly, contact area manager or supervisor before proceeding.
- 6.3.3 Aseptic Addition(s)
 - 6.3.3.1 For aseptic additions, use a tubing welder to make necessary connections to the BPR specified addition line.
 - 6.3.3.2 After successfully tubing welding the addition port tubing to the addition container, open the clamp on the addition leg. This will allow the flow of growth media or other addition material to enter the vessel.
 - 6.3.3.3 Pump the material into the vessel using a peristaltic pump. When addition is complete, close the clamp on the vessel.
 - 6.3.3.4 The addition port tubing may be re-used for another aseptic addition by utilizing a tubing weld to connect more addition containers.
 - 6.3.3.5 Alternatively, a tubing sealer may be used to seal the addition port tubing. The tubing seal may be cut in half to separate the port tubing from the last addition container. The sterile side of the vessel bag port will now be isolated and ready for later additions required by the BPR.
- 6.3.4 Aseptic Sampling
 - 6.3.4.1 Carry out the sterile removal of a sample via the sampling port. Remove the cap from theclave connector of the sampling port and store the cap for the duration of the sampling so that it may be used again.
 - 6.3.4.2 Disinfect the clave connector by spraying with an approved disinfectant solution (typically 70% IPA).

- 6.3.4.3 Obtain 2 sterile Luer Lock syringes for sampling (or other Luer Lock fitted container.) The first sampling syringe will be used to remove any hold-up volume from the sampling tubing. The second sampling syringe will be utilized to remove a representative sample for in process testing.
- 6.3.4.4 Remove the packaging from the sterile sampling syringe.
- 6.3.4.5 Position the syringe and tighten by turning it clockwise until it stops against the sampling port clave connector.
- 6.3.4.6 Open the tube clamp of the sampling tube, fill the sampling syringe once to remove the hold-up volume, then close the tube clamp on the sampling tube. Turn the sampling syringe counterclockwise and remove.
- 6.3.4.7 Dispose of any sample syringes per **SOP 19407 -Flow of Personnel, Materials, Equipment, and Waste in the CGMP Areas of the ATRF.**
- 6.3.4.8 Repeat the procedure above for the vessel sample collection with the second sterile syringe and remove the BPR specified sample volume.
- 6.3.4.9 Measure the collected samples quickly to minimize the effects of degassing when pH testing is performed.
- 6.3.4.10 At the completion of sampling spray the clave connector with 70% IPA (or other approved disinfectant) and place the sampling line cap onto connector.
- 6.3.5 System Shutdown
 - 6.3.5.1 At the completion of a production process, verify that the vessel bag contents have been removed via drain port. End the MFCS batch run at the local computer.
 - 6.3.5.2 Place all control loops (ie. Sparge, Overlay, Temperature, Agitation) to OFF mode and allow the vessel bag to deflate as the inlet air returns to 0 lpm. Deactivate the currently in-use vessel at the DCU touch panel.
 - 6.3.5.3 Once the bag has deflated and the agitator has stopped rotating, open the bag holder door. Remove the agitator clamp from the magnetic drive coupling. The bag will drop slightly after this has been completed. Place the vessel drop valve in the open position and remove the drain valve retention collar; place the collar in a safe location for re-use.
 - 6.3.5.4 Remove any connected reusable pH/DO sensors from the bag, clean and store as appropriate.

- 6.3.5.5 Disconnect all air filters from the filter holder and allow them to rest on the top of the vessel bag.
- 6.3.5.6 Remove the vessel bag from the bag holder. Disconnect the inlet and exhaust filters and retain for integrity testing. Discard of the vessel bag as appropriate per **SOP 19407 - Flow of Personnel, Materials, Equipment, and Waste in the CGMP Areas of the ATRF.**
- 6.3.5.7 To switch on the control / supply unit, turn the main switch [Mains I/O] on the control / supply unit to the "I" position.
- 6.3.5.8 To switch on the bag holder, turn the main switch [Mains I/O] of the bag holder to the "I" position.
- 6.3.5.9 Press the switch [Unit 1 off/on] and/or [Unit 2 off/on] on the control / supply unit to turn on the bag holder's measurement and control system.
- 6.3.5.10 Turn off all vessel process utilities at the utility panel.
- 6.3.5.11 Wipe down the outside of the bag holder with 70% IPA (or equivalent disinfectant). Ensure to not spray critical components like the optical pH/DO sensors or pressure switches. There may be adverse effects from the disinfecting agent.

7.0 Documentation

- 7.1 Document the performance of this procedure on the BPR and in the equipment logbook.

8.0 References and Related Documents

- 8.1 **SOP 12118** *Maintenance and Storage of Dissolved Oxygen and pH Probes*
- 8.2 **SOP 19407** *Flow of Personnel, Materials, Equipment, and Waste in the CGMP Areas of the ATRF*
- 8.3 **SOP 21531** *Equipment Logs*