

SOP: Applikon ez-Control Bioreactor Controller Operation

Approvals

Preparer: John Buford

Date: 19DEC13

Reviewer: Jason McMillan

Date: 16SEP14

Reviewer: Hetal Doshi

Date: 8JULY17

Reviewer: Dr. Maggie Bryans

Date: 10JULY17

1. Purpose

- 1.1. Cultivate a cell culture using an Applikon ez-Control bioreactor controller and a 3-liter glass autoclavable bioreactor.

2. Scope and Applicability

- 2.1. A bioreactor controller is used to measure and control process variables (temperature, pH, dissolved oxygen, stirrer speed, and so on) within a bioreactor vessel such as a glass autoclavable bioreactor, a single use bioreactor, or a single use wave bag. This SOP provides the basic steps required to cultivate a cell culture using the Applikon ez-Control and a 3-liter glass autoclavable bioreactor. Other process SOPs are intended to provide additional details such as culture medium composition and volume, control process settings, and run time.

3. Summary of Method

- 3.1. Preparing the controller
 - 3.1.1. Power up the controller and login
 - 3.1.2. Enter the project name (optional)
 - 3.1.3. Emergency stop and resume (emergency only)
- 3.2. Preparing the bioreactor
 - 3.2.1. Fill the bioreactor with culture medium or 100ml 1X PBS
 - 3.2.2. Calibrate the pH sensor
 - 3.2.3. Mount sensors to the bioreactor
 - 3.2.4. Prepare the liquid addition bottles
 - 3.2.5. Mount connections to the bioreactor
 - 3.2.6. Autoclave the bioreactor and addition bottles
- 3.3. Connecting the bioreactor to the controller
 - 3.3.1. Verify that all control loops are switched off
 - 3.3.2. Connect the sensors
 - 3.3.3. Connect the heating blanket
 - 3.3.4. Connect the aeration
 - 3.3.5. Connect the stirrer motor
 - 3.3.6. Connect the alkaline bottle
 - 3.3.7. Enter process parameter settings
 - 3.3.8. Start all control loops
 - 3.3.9. Calibrate the DO sensor
 - 3.3.10. Reset dose monitor values

SOP: Applikon ez-Control Bioreactor Controller Operation

- 3.4. Starting the cultivation
 - 3.4.1. Start process data acquisition
 - 3.4.2. Inoculate the bioreactor
- 3.5. Monitoring the cell culture
 - 3.5.1. View process data
 - 3.5.2. Sample the cell culture
- 3.6. Harvesting the cell culture
 - 3.6.1. Stop the controllers
 - 3.6.2. Save process data
 - 3.6.3. Disconnect the bioreactor
 - 3.6.4. Decant the cell culture
- 3.7. Cleaning the bioreactor
 - 3.7.1. Clean the sensors
 - 3.7.2. Pre-clean the bioreactor in place
 - 3.7.3. Disassemble the bioreactor and clean all parts
 - 3.7.4. Clean the porous sparger tip (optional)

4. References

- 4.1. ez-Control Hardware Manual for Autoclavable Bioreactors, Applikon Biotechnology, Hardware version 2, Documentation version 1.10.
- 4.2. ez-Control Operator Manual for Autoclavable Bioreactors, Applikon Biotechnology, Software version 1.6X, Documentation version 1.0.
- 4.3. ez-Control Software Reference Manual, ez-Control for Autoclavable and Single Use Applications, Applikon Biotechnology, Software version 1.6X, Documentation version 1.0.
- 4.4. BioXpert Lite Data Acquisition Program User Manual, Applikon Biotechnology, Software version 1.1X, February 2010.
- 4.5. Labconco Purifier Class 2 Biosafety Cabinet SOP

5. Precautions

- 5.1. Alkaline solutions used for controlling pH are caustic. Read the Material Safety Data Sheet (MSDS) for hazards, handling and storage information. Wear personal protection equipment (PPE).
- 5.2. Do not put the stirrer motor or the heating blanket in the autoclave.
- 5.3. The O₂ electrolyte solution used in the DO probe is a strong Alkaline (pH13) solution. Avoid contact with skin, mucous membrane, or eyes. If contact does occur, flush the affected area with water.

6. Responsibilities

- 6.1. It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as described and to update the procedure when necessary.
- 6.2. It is the responsibility of the students/technician to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.

SOP: Applikon ez-Control Bioreactor Controller Operation

7. Equipment and Materials

- 7.1. Applikon 3-liter glass autoclavable bioreactor:
 - 7.1.1. Vessel
 - 7.1.2. Head plate
 - 7.1.3. Gas sparger (pre-mounted on the head plate)
 - 7.1.4. Impeller (pre-mounted on the head plate)
 - 7.1.5. Air outlet condenser (pre-mounted on the head plate)
 - 7.1.6. Septum (pre-mounted on the head plate)
 - 7.1.7. Sample pipe (pre-mounted on the head plate)
 - 7.1.8. pH sensor
 - 7.1.9. DO sensor
 - 7.1.10. Temperature sensor
 - 7.1.11. Stirrer motor
 - 7.1.12. Heating blanket
- 7.2. Applikon ez-Control bioreactor controller
- 7.3. Laboratory gasses:
 - 7.3.1. Air compressor
 - 7.3.2. O₂ (optional)
 - 7.3.3. CO₂
- 7.4. pH 4.0 and pH 7.0 buffer standards
- 7.5. Liquid addition bottles
- 7.6. Sterile sample bottle (Chemglass catalog#CLS-1437-B)
- 7.7. Alkaline solution (generally 1M sodium bicarbonate)
- 7.8. Gas filters, 0.2 µm
- 7.9. Autoclavable silicone tubing, size 14 (1.6 mm interior diameter)
- 7.10. Autoclavable silicone tubing, size 16 (3.1 mm interior diameter)
- 7.11. Autoclavable silicone tubing, size 25 (4.8 mm interior diameter)
- 7.12. Tubing clamps
- 7.13. Cotton and aluminum foil (for autoclaving)
- 7.14. Autoclave indicator tape
- 7.15. Culture medium
- 7.16. Culture inoculum
- 7.17. Autoclave (such as a Yamato Sterilizer SM501)
- 7.18. Computer system with BioXpert Lite installed
- 7.19. Autoclavable male and female connectors

8. Procedure

8.1. Preparing the controller

8.1.1. Power up the controller and login

The controller is used to measure and control process variables (temperature, pH, dissolved oxygen, and stirrer speed) within a bioreactor. After switching on the power

SOP: Applikon ez-Control Bioreactor Controller Operation

of the controller, it presents itself (after initialization) on the touch screen display with its Home screen, which is generally configured as the Synoptic View shown in Figure 2. The operator uses the touch screen display to monitor the bioreactor conditions and to enter process control parameters. Four authorization (login) levels allow access to various controller capabilities: View (initial level), Operator, System engineer, and Service engineer level. The View authorization level only allows process values to be monitored. To set process control parameters, the user needs to login as Operator. For an example of how to navigate the controller screens, see Example 1.

- 8.1.1.1. Power up the controller using the green power switch located on the back of the controller (upper right).
- 8.1.1.2. The touch screen display located on the front shows that the controller is initializing. Once initialization completes, the display switches to the Home screen.
- 8.1.1.3. Login as Operator using the touch screen display:
 - 8.1.1.3.1. Home > login button (top middle) > Operator > Login
 - 8.1.1.3.2. Enter the Operator password: 0000
- 8.1.1.4. To logout: Home > login button (top middle) > View *Note: logout is automatic after a period of inactivity (generally 10 minutes).*
- 8.1.1.5. The touch screen backlight is switched off automatically after a period of inactivity (generally 30 minutes) and the touch screen goes dark. To switch on the backlight, touch the screen.

8.1.2. Enter the project name (optional)

The project name is displayed on the top of all controller screens in order to identify the bioreactor system and cell culture being cultivated.

- 8.1.2.1. Login as Operator per section 8.1.1.3 if not already logged in.
- 8.1.2.2. Home > project name button (top left)
- 8.1.2.3. Enter a descriptive name

8.1.3. Emergency stop and resume (emergency only)

The Emergency Stop button on the front of the control shuts down all control loops immediately and displays the Emergency Stop screen. Measurements continue to be collected.

- 8.1.3.1. In the event of an emergency requiring the bioreactor process to stop immediately, press the red Emergency Stop button located on the front of the controller.
- 8.1.3.2. To resume the bioreactor process:
 - 8.1.3.2.1. Remove the cause of the Emergency Stop.
 - 8.1.3.2.2. Turn the Emergency Stop button clockwise until it resets itself to the normal position.
 - 8.1.3.2.3. Login as System Engineer.
 - 8.1.3.2.4. Verify that the bioreactor is in a safe condition.

SOP: Applikon ez-Control Bioreactor Controller Operation

8.1.3.2.5. Select Resume on the Emergency Stop screen to restart the control loops.

8.2. Preparing the bioreactor

8.2.1. Fill the bioreactor with culture medium

The bioreactor is filled with culture medium for bacterial cell culture before it is autoclaved (if it will not damage the media) so that the medium is sterilized along with the bioreactor. Do not exceed the working volume of the bioreactor (2.4 liters for a 3-liter bioreactor). Leave enough space for inoculation and nutrients to be added during cultivation. For mammalian cell culture the media is added to the bioreactor after autoclaving. 100 ml of 1XPBS is added to the bioreactor before autoclaving.

- 8.2.1.1. A process SOP should provide details regarding culture medium composition and volume. E.g., refer to section 8.5 of Batch Culture of anti-IL-8 Monoclonal Antibody secreting CHO DP-12 Cells
- 8.2.1.2. Loosen the six mill nuts that fastens the head plate on the bioreactor vessel and remove the head plate.
- 8.2.1.3. Inspect the integrity of the large O-ring on the vessel stand and headplate. Replace if worn or crack.
- 8.2.1.4. Assemble the headplate
 - 8.2.1.4.1. Inspect the integrity of the O-rings on the harvest tube, sparger and the thermowell.
 - 8.2.1.4.2. Attach a sample tube, and a thermowell. Attach the sparger tube in such a way that the tube is aligned beneath the stirrer impeller.
- 8.2.1.5. Add 100 ml of 1X PBS to the bioreactor vessel
- 8.2.1.6. Mount the head plate on top of the vessel and fasten with the six mill nuts finger-tight.
- 8.2.1.7. Assemble the topside of the head plate
 - 8.2.1.7.1. Inspect the integrity of the O-ring in the condenser port
 - 8.2.1.7.2. Inspect the black seal at the bottom of the condenser underneath the retainer nut. Replace if worn or cracked.
 - 8.2.1.7.3. Attach the condenser to the head plate by placing the condenser into the condenser port making sure that the barbed connectors are facing out.

8.2.2. Calibrate the pH sensor

Before the bioreactor is autoclaved, the pH (acidity) sensor needs to be calibrated with pH 4.0 and pH 7.0 buffer standards in order to obtain accurate measurement values during cultivation.

- 8.2.2.1. Measure (and record in Batch Record) the temperature of the pH buffer standards using a thermometer.

SOP: Applikon ez-Control Bioreactor Controller Operation

- 8.2.2.2. Remove the protective cap from the bottom of the pH sensor. Rinse the pH sensor with de-ionized water and pat it dry with a clean lint-free laboratory wipe.
- 8.2.2.3. Remove the pH sensor screw cap. Connect the pH sensor to the pH sensor cable on the right side of the controller. Verify that the pH sensor cable is plugged into the controller correctly.
- 8.2.2.4. Login as Operator per section 8.1.1.3 if not already logged in.
- 8.2.2.5. Go to the controller pH Settings screen: Home > pH (bottom)
- 8.2.2.6. Verify that the pH control loop is off (i.e. the pH Process Value button is grey or yellow, not green). If it is on, touch the button Stop pH controller.
- 8.2.2.7. Touch the button Calibrate pH to go to the pH Calibration screen. The numerical data for Slope, Offset and any Sample correction are displayed.
- 8.2.2.8. Touch reset calibration value. Touch yes, when prompted are you sure?
- 8.2.2.9. Touch the button 2-point calibration.
- 8.2.2.10. Enter the temperature of the buffer solutions using the numeric keypad.
- 8.2.2.11. When prompted for the pH value of the first buffer solution, put the pH sensor in the pH 4.0 buffer standard and wait until the shown process value stabilizes (shown near the Cancel button). Enter the pH value using the numeric keypad.
- 8.2.2.12. Rinse the pH sensor and repeat using the pH 7.0 buffer standard. Again, wait for the shown process value to stabilize and enter the corresponding pH.
- 8.2.2.13. Record slope and offset in the Batch Record.
- 8.2.2.14. Verify the slope and offset is within the expected value. Refer below for the expected values
Slope Expected Value: 0.95-1.05
Offset Expected Value: $< \pm 0.3$
- 8.2.2.15. If the slope and offset value are out of range, repeat the calibration steps.

8.2.3. Mount sensors to the bioreactor

pH and DO sensors are mounted to the bioreactor before it is autoclaved so that the sensors are sterilized along with the bioreactor. Some systems also use a foam / level sensor.

- 8.2.3.1. Disconnect the cable of the pH sensor.
- 8.2.3.2. Cover the pH sensor connector with the pH sensor screw cap. Verify that the rubber gasket is in place between the sensor connector and the cap.
- 8.2.3.3. Insert the pH sensor into its port in the head plate and fasten it. See Figure 6 for the location of the pH sensor port.
- 8.2.3.4. Remove the protective cap from the bottom of the DO sensor.
- 8.2.3.5. Inspect the screen at the bottom of the probe tip. Replace if damaged.

SOP: Applikon ez-Control Bioreactor Controller Operation

- 8.2.3.6. Holding the probe in a vertical position, unscrew module from the bottom of the probe
- 8.2.3.7. Inspect the integrity of the O-ring underneath the module and replace if worn or cracked
- 8.2.3.8. Replenish DO electrolyte solution. There should be 1 ml of electrolyte solution in the membrane module.
- 8.2.3.9. Screw the membrane module to the probe
- 8.2.3.10 Repeat steps 8.2.3.2 and 8.2.3.3 for the DO sensor.

8.2.4. Prepare the liquid addition bottles

Liquid addition bottles are used to add liquids to the bioreactor aseptically (see Figure 7). For example, Media is added, alkaline solution is added to raise pH and inoculum is added to begin cultivation. Addition bottles are sterilized along with the bioreactor.

- 8.2.4.1. A process SOP should provide the information regarding the alkaline solution composition and water.
- 8.2.4.2. Fill a liquid 250ml addition bottle with alkaline solution, no more than 2/3 (150ml) full so that it can be autoclaved. Cap the alkaline bottle with a two-port top.
- 8.2.4.3. Connect the air inlet on the alkaline bottle to a gas filter using a short length (approx. 7 cm) of size 25 tubing. Do not clamp.
- 8.2.4.4. Add 5 mL laboratory grade water to a 500ml addition bottle to be used for transferring inoculum. This will improve the heat transfer during sterilization in the autoclave. Cap the inoculum transfer bottle with a two-port top.
- 8.2.4.5. Connect the air inlet on the inoculum transfer bottle to a gas filter using a short length (approx. 7 cm) of size 25 tubing. Do not clamp.
- 8.2.4.6. Connect the liquid outlet on the inoculum transfer bottle to an autoclavable male and female connector using a long length (approx. 75 cm) of size 25 tubing.
- 8.2.4.7. Cover the gas filters and autoclavable male connector loosely with aluminum foil.
- 8.2.4.8 Repeat steps 8.2.4.4. to 8.2.4.7 with 1L media addition bottle.

8.2.5. Mount connections to the bioreactor *Gas filters and silicone tubing connections are mounted to the bioreactor before it is autoclaved so that they are sterilized along with the bioreactor. See section 7 for the silicone tubing size specifications.*

- 8.2.5.1. Connect one of the medium inlet triplet nipples to a second triplet nipple using a short length (approx. 7 cm) of size 14 tubing. Connect a medium length (approx. 15 cm) of size 14 tubing to the third medium inlet triplet nipple. Clamp the tubing closed.

SOP: Applikon ez-Control Bioreactor Controller Operation

- 8.2.5.2. Connect the addition pipe to the liquid outlet on the alkaline bottle using an extra-long length (approx. 75 cm) of size 25 tubing. Clamp the tubing closed.
- 8.2.5.3. Connect a medium length (approx. 15 cm) of size 16 tubing to the sample pipe. Clamp the tubing closed.
- 8.2.5.4. Connect the sparger inlet to a gas filter using approx. 60 cm length of size 25 tubing. Clamp the tubing closed
- 8.2.5.5. Connect the bottom condenser nipple on the middle condenser nipple using a medium length (approx. 15 cm) of size 25 tubing.
- 8.2.5.6. Connect the top condenser nipple to a gas filter using approx. 50 cm of size 25 tubing. Do not clamp.
- 8.2.5.7. Insert a septum into its holder in the head plate and fasten it.
- 8.2.5.8. Close all the tubing ends with cotton and cover the ends with autoclavable aluminum foil.
- 8.2.5.9. Verify that the gas filter connected to the condenser(air outlet) is open to avoid pressure differences during autoclaving. Cover the gas filters loosely with aluminum foil.
- 8.2.5.10. Verify the mountings of all nipples and other auxiliaries.
- 8.2.5.11. Cover head plate with autoclavable aluminum foil

8.2.6. Autoclave the bioreactor and liquid addition bottles

The assembled bioreactor is autoclaved before cultivation in order to create a sterile environment inside the bioreactor. Do not autoclave the heating blanket or the stirrer motor. Single use bioreactors are not autoclaved; they are sterilized by the manufacturer.

- 8.2.6.1. Apply autoclave indicator tape to the aluminum foil on the alkaline bottle and the inoculum transfer bottle.
- 8.2.6.2. Place the assembled bioreactor, the alkaline bottle without disconnecting tubing, 1L addition bottle with tubing and 500ml Inoculum transfer bottle with tubing in the autoclave.
- 8.2.6.3. Loosen the caps on the alkaline bottle, liquid addition bottle and the inoculum transfer bottle.
- 8.2.6.4. Close the autoclave and select the sterilization temperature at 121°C for 20 minutes.
- 8.2.6.5. When the cycle completes, allow the autoclave to cool gradually. Do not open the autoclave until the temperature in the autoclave has dropped below 90°C. After reaching that temperature, open the autoclave to allow it to cool down until the contents can be unloaded safely.
- 8.2.6.6. Tighten the caps on the alkaline bottle and the inoculum transfer bottle.
- 8.2.6.7. Remove the assembled bioreactor and the alkaline bottle together and place beside them on the right side of the controller without disconnecting the tubing.

SOP: Applikon ez-Control Bioreactor Controller Operation

8.2.6.8. Remove the inoculum transfer bottle and place it in a biological safety cabinet.

8.2.6.9. Allow to cool to room temperature.

8.2.6.10. Perform a visual inspection to verify that the autoclave indicator tape changes color and that the bioreactor is dry.

8.2.6.11. Remove aluminum foil from gas filters on the bioreactor and alkaline bottle. Leave the foil on the inoculum transfer bottle and the addition bottle.

8.2.6.12. Place the bioreactor and alkaline bottle on the right side of the controller.

8.3. *Connecting the bioreactor to the controller*

After the assembled bioreactor is autoclaved, it must be connected to the controller.

8.3.1. *Verify that all control loops are switched off*

8.3.1.1. Login as Operator per section 8.1.1.3 if not already logged in.

8.3.1.2. Verify that all control loops are switched off. The Process Value buttons (bottom of Home Screen) should be gray or yellow. If necessary, stop controllers: Home > Menu (top right) > Start/Stop all controllers > Stop all controllers

8.3.2 *Addition of the media to the Bioreactor*

If mammalian cells are used for the cell culture production bioreactor is autoclaved without addition of the media. The media is added to the bioreactor using the 1L addition bottle.

8.3.2.1. Refer to the Batch culture SOP for the details regarding the media composition

8.3.2.2. Spray or swab the required material with 70% ethanol, and place in the biological safety cabinet

8.3.2.3. Aseptically add the media component to the prepared 1L addition bottle with the tubing and autoclavable male connector attached.

8.3.2.4. Be sure the cap is on tightly and remove the 1L addition bottle with tubing and autoclavable male connector attached and bring it over to Applikon bioreactor.

8.3.2.5. Carefully remove the foil from the female connector on the sample port of the Applikon bioreactor.

8.3.2.6. Carefully remove the aluminum foil from the male and female connector on the 1L addition bottle and connect the male female connector on the sample port tubing of the bioreactor.

8.3.2.7. Open the clamp on the female connector on the addition port of the Applikon bioreactor.

8.3.2.8. On the Applikon reactor touch screen select Menu. Manual Control > Acid Pump On.

8.3.2.9. As the pump turns feed the tubing around it. Use care to avoid pinching fingers.

SOP: Applikon ez-Control Bioreactor Controller Operation

8.3.2.10. Once all of the media has been transferred into the vessel, turn off the acid pump. On the Applikon screen select Menu > Manual control > Acid Pump off.

8.3.2.11. Disconnect the male connector of the addition bottle from the female connector on the sample port of the bioreactor. Bend the tubing of the addition port and reattach the clamp.

8.3.3. Connect the sensors

Sensors are connected electrically to the controller.

8.3.3.1. Place the bioreactor and alkaline bottle on the right side of the controller if they are not already.

8.3.3.2. Remove the pH sensor screw cap. Connect the pH sensor to the pH sensor cable on the right side of the controller. Verify that the pH sensor cable is plugged into the controller correctly.

8.3.3.3. Remove the DO sensor screw cap. Connect the DO sensor to the DO sensor cable on the right side of the controller. Verify that the DO sensor cable is plugged into the controller correctly.

8.3.3.4. Insert the temperature sensor into the thermometer pocket. Verify that the temperature sensor cable is plugged into the controller correctly. Fill the thermometer pocket with MilliQ water in order to decrease the dead time of the sensor and make temperature control more accurate.

8.3.4. Connect the heating blanket

An electric heating blanket is used for warming the bioreactor as needed. Some systems also use a thermo circulator and cooling water for cooling the bioreactor.

8.3.4.1. Wrap the heating blanket around the bioreactor vessel (around the glass and inside the support legs). Position the blanket so that the volume markings on the vessel are visible. Fasten the blanket in place using the Velcro ends of the blanket.

8.3.4.2. Verify that the heating blanket is plugged into the controller correctly.

8.3.5. Laboratory gasses (air, O₂, and CO₂) are added by the controller to the bioreactor sparger in order to control DO and to lower pH as needed.

8.3.5.1. Connect the aeration outlet of the controller to the gas filter on the bioreactor sparger inlet using size 16 tubing.

8.3.5.2. Open the CO₂ tank and set its regulator to 10 psi.

8.3.6. Connect the stirrer motor

The stirrer motor and the impeller are used to control agitation of the cell culture. When connecting the stirrer motor, it is helpful for the stirrer to be on.

8.3.6.1. Login as Operator per section 8.1.1.3 if not already logged in.

8.3.6.2. Go to the Stirrer Settings screen: Home > Stirrer (bottom left)

8.3.6.3. Touch the alarm limit button > Lower alarm limit > enter 58. > Touch the stirrer set point, enter 60.

8.3.6.4. Start the stirrer: touch the button Start Stirrer controller.

SOP: Applikon ez-Control Bioreactor Controller Operation

8.3.6.5. Position the stirrer motor vertically over the bioreactor head plate and slowly lower it into place. Verify that the impeller is turning.

8.3.6.6. Stop the stirrer: touch the button Start Stirrer controller.

8.3.7. Connect the alkaline bottle

A peristaltic pump is used to dispense measured amounts of alkaline solution from an addition bottle to the bioreactor. The silicone tubing that connects the alkaline bottle to the bioreactor needs to be threaded through the pump. See Figure 8.

8.3.7.1. Login as Operator per section 8.1.1.3 if not already logged in.

8.3.7.2. Locate the alkaline pump on the right front panel of the controller. Open the pump cover.

8.3.7.3. Locate the tubing that connects the alkaline bottle to the bioreactor. Bend the middle of the tubing into a U shape and hold in one hand. Clip the tubing U into the lower pump clamp.

8.3.7.4. Turn the pump on manually: Home > Menu > Manual control>Alkaline pump: On

8.3.7.5. Ease the tubing into the pump as the pump rotors are turning. Use care to avoid pinching fingers.

8.3.7.6. Turn the pump off.

8.3.7.7. Clip the tubing into the upper pump clamp.

8.3.7.8. Close the pump cover.

8.3.7.9. Turn the pump on manually and watch the solution being drawn from the bottle into the tubing. When the solution reaches the bioreactor, turn the pump off.

8.3.8. Enter process parameter settings

The pH, temperature, DO, and stirrer control loops should be adjusted to desired process parameter settings. Each control loop has settings for upper alarm limit, lower alarm limit, and setpoint. Additional settings for PID controls may also be set at this time.

8.3.8.1. A process SOP should provide details regarding process setpoints and alarm limits.

8.3.8.2. Login as Operator per section 8.1.1.3 if not already logged in.

8.3.8.3. Go to the pH Settings screen: Home > pH (bottom)

8.3.8.4. Enter the process settings for the pH control loop:

8.3.8.4.1. Enter the pH upper alarm limit: pH settings > Alarm limits > Upper alarm limit Enter the pH upper alarm limit value provided by the process SOP.

8.3.8.4.2. Enter the pH lower alarm limit: pH settings > Alarm limits > Lower alarm limit Enter the pH lower alarm limit value provided by the process SOP.

8.3.8.4.3. Enter the pH setpoint: pH settings > pH setpoint Enter the pH setpoint value provided by the process SOP.

SOP: Applikon ez-Control Bioreactor Controller Operation

8.3.8.4.4. Enter any additional pH PID controls provided by the process SOP: pH settings > pH controller setup

8.3.8.5. Repeat for temperature and stirrer control loops.

8.3.8.5.1. For the stirrer control loop, change the set point from 60 to 73, then change the lower limit.

8.3.9. Start the pH controller loop, temperature controller loop and Stirrer controller loop. Don't start the DO controller Loop until the DO probe is calibrated. The DO sensor needs to be polarized for at least 6 hours and the temperature and pH need to be stabilized at set point before the DO sensor can be calibrated.

8.3.9.1. Login as Operator per section 8.1.1.3 if not already logged in.

8.3.9.2. Start pH control loop: Home > pH (bottom)>Start pH control loop

8.3.9.3. Start temp control loop: Home > temp (bottom) >Start temp Loop

8.3.9.4. Start Stirrer control loop: Home> stirrer (bottom)> Start stirrer Loop.

8.3.9.5. Allow the process to run for at least 6 hours.

8.3.10. Calibrate the DO sensor

The DO measurement is based on the polarographic principle (Clark-cell). Therefore, the sensor must be polarized for at least 6 hours before it can be calibrated.

8.3.10.1. Start the air pump. Set the pressure at 10 psi.

8.3.10.2. Login as Operator per section 8.1.1.3 if not already logged in.

8.3.10.3. Verify that the medium in the bioreactor is stable at process temperature.

8.3.10.4. Stop the DO control loop: Home > DO (bottom) > Stop DO controller

8.3.10.5. Go to the DO Calibration screen: Home > DO > Calibrate DO The numerical data for Slope and Offset are displayed.

8.3.10.6. Verify if the measuring range is set to Air. If not, set it for air: Calibrate DO > Set measurement range > Measurement range for air

8.3.10.7. Open the aeration valve manually: Home > DO > Manual control > O2 Valve: On

8.3.10.8. Continue aeration until DO reading is stable (15 to 20 minutes).

8.3.10.9. Close the aeration valve manually: Home > DO > Manual control > O2 Valve: Off

8.3.10.10. Set the DO calibration value to 100%: Home > DO > Calibrate DO > Calibrate > Enter Calibration Value > 100

8.3.10.11. Return to the DO Calibration screen to verify the newly found calibration data (slope and offset). The expected slope value of the sensor (for measurement range for air) is:

2.0 to 4.0 at 25 °C

1.5 to 3.0 at 37 °C

8.3.10.12. Start the DO control loop: DO (bottom) > Start DO controller.

8.3.11. Reset dose monitor values

When all control loops are at set-point, the bioreactor system is ready for cultivation (inoculation). All Dose Monitor values should be reset to 0 ml.

SOP: Applikon ez-Control Bioreactor Controller Operation

8.3.11.1. Go to the Home screen and verify that the control loops are on. Allow the process to run until all control loops are at set-point.

8.3.11.2. Reset all dose monitor values: Home > Menu > Dose Monitor > Reset all dose monitors > Are you sure? Yes

8.4. Starting the cultivation

8.4.1. Inoculate the bioreactor

Once the process parameters in the bioreactor are at their setpoints, the inoculum is added to the bioreactor aseptically. This SOP makes use of a sterile addition bottle for this purpose.

8.4.1.1. Fill the sterile inoculum transfer bottle with inoculum using aseptic technique per the biosafety cabinet SOP. Do not remove the foil from the gas filter or the autoclavable male connector. Recap the bottle before removing it from the biosafety cabinet. Place the inoculum bottle on the right side of the controller.

8.4.1.2. Login as Operator per section 8.1.1.3 if not already logged in.

8.4.1.3. Stop all control loops: Home > Menu > Start/Stop all controllers > Stop all controllers

8.4.1.4. Swab gloves with 70% isopropanol; spray the female connector on the sample port.

8.4.1.5. Remove the foil from the attached tubing connected to the male connector and the gas filter on the inoculum transfer bottle. Connect the female connector on the sample port of the bioreactor to the male connector of the inoculum bottle.

8.4.1.6. Remove the clamp on the female connector on the addition port of the Applikon bioreactor.

8.4.1.7. On the Applikon bioreactor touch screen select Menu. Manual Control > Acid Pump ON

8.4.1.8. As the pump turns, feed the tubing around it. Use care to avoid pinching fingers.

8.4.1.9. Once all of the cell suspension has been transferred into the vessel, turn off the acid pump. On the Applikon screen select Menu > Manual control > Acid pump Off

8.4.1.10. Disconnect the male connector of the addition bottle from the female connector on the sample port of the bioreactor. Bend the tubing of the addition port and reattach the clamp.

8.4.1.11. Re-start all control loops: Home > Menu > Start/Stop all controllers > Start all controllers.

8.5. Monitoring the cell culture

8.5.1. View process data

Process values for the last 72 hours are stored in controller memory and can be displayed using the Trend View screen. Process values for the entire run are stored and displayed using BioXpert Lite.

SOP: Applikon ez-Control Bioreactor Controller Operation

8.5.1.1. To view process data for a specific process parameter using the Trend View:

- 8.5.1.1.1. Login as Operator per section 8.1.1.3 if not already logged in.
- 8.5.1.1.2. Home > process value button (2nd row from the bottom)
- 8.5.1.1.3. Touch the X-axis button (bottom left) in order to change the range of the time axis (between 1 and 72 hours).
- 8.5.1.1.4. Touch the Y-axis upper limit button (top right) or lower limit button (bottom right) in order to change the range of the process value axis.

8.5.2. Sample the cell culture

Periodically, the cell culture is sampled in order to measure cell concentration and perform product assays per the process SOP. Some bioreactors include a sample system connected to the sample pipe which enables cell culture to be drawn into a sample bottle that can then be replaced aseptically. This system uses a sample bottle to draw cell culture from the sample pipe.

- 8.5.2.1. Refer to the Batch Culture SOP for sample to be withdrawn.
- 8.5.2.2. Log in to Applikon EZ Controller as operator per Applikon Operator SOP
- 8.5.2.3. Raise the stirrer upper limit to 150 rpm
- 8.5.2.4. Change the stirrer setting to 125 rpm.
- 8.5.2.5. Spray the head plate near the sampling tube with 70% IPA.
- 8.5.2.6. Open the white clamp such that it allows the liquid to flow
- 8.5.2.7. Connect the sample port tubing to sterile sample bottle inlet one (long inlet tube). Connect a size 16 tubing to sample bottle inlet 2. Connect a 60ml syringe to the sample port tubing and draw back the plunger until approximately 10ml of cell culture enters the sample bottle
- 8.5.2.8. Disconnect the sample port tubing from the sample bottle.
- 8.5.2.9. Close the clamp
- 8.5.2.10. Change the stirrer setting to 75 rpm.
- 8.5.2.11. Change the stirrer upper limit back to 76 rpm
- 8.5.2.12. Use this sample for cell counts and product assays per the process SOP.

8.6. Harvesting the cell culture

A process SOP should specify when the cell culture is to be harvested.

8.6.1. Stop the controllers

- 8.6.1.1. Login as Operator per section 8.1.1.3 if not already logged in.
- 8.6.1.2. Stop all control loops: Home > Menu > Start/Stop all controllers > Stop all controllers
- 8.6.1.3. Close the CO2 tank.
- 8.6.1.4. Close the air tank.

8.6.2. Disconnect the bioreactor

The bioreactor must be disconnected from the controller so that the bioreactor can be decanted.

SOP: Applikon ez-Control Bioreactor Controller Operation

- 8.6.2.1. Locate the tubing that connects the alkaline bottle to the bioreactor. Clamp the tubing near the bioreactor. Disconnect the tubing from the alkaline bottle and remove the tubing from the controller pump.
- 8.6.2.2. Lift the stirrer motor from the bioreactor head plate and set the motor aside.
- 8.6.2.3. Disconnect the gas filter on the bioreactor sparger inlet from the tubing to the aeration outlet of the controller.
- 8.6.2.4. Unwrap the heating blanket from around the bioreactor vessel and set the blanket aside being sure it is lying flat.
- 8.6.2.5. Disconnect the pH sensor cable from the pH sensor. Cover the pH sensor connector with the pH sensor screw cap.
- 8.6.2.6. Repeat step 8.6.2.5 for the DO sensor.

8.7. *Cleaning the bioreactor*

8.7.1. *Clean and store the sensors*

- 8.7.1.1. Remove pH and DO sensors from the bioreactor head plate.
- 8.7.1.2. Rinse the pH and DO sensors thoroughly with MilliQ water, being careful to remove all broth-residue. Gently pat dry with a clean lint-free laboratory wipe. Spray with 70% IPA and gently pat dry with a clean lint-free laboratory wipe
- 8.7.1.3.. Rinse with MilliQ water and pat dry with a clean lint-free laboratory wipe.
- 8.7.1.4. Fill the protective cap of the pH sensor 1/2 full with 3M potassium chloride (KCl) solution. Cover the tip of the pH sensor with its protective cap. Verify that the pH electrode is completely immersed in KCl solution.
- 8.7.1.5. Cover the tip of the DO sensor with its protective cap. The DO sensor can be stored in an electrolyte solution upright for a short term. For long term storage, store dry.
- 8.7.1.6. Cover the pH sensor connector with the pH sensor screw cap. Repeat for the DO sensor.

8.7.2. *Decant the cell culture*

- 8.7.2.1. Loosen the six mill nuts that fasten the head plate on the bioreactor vessel and remove the head plate.
- 8.7.2.3. Transfer the cell culture into 250 ml autoclaved centrifuge bottles by pipetting with 25 ml or 50 ml serological pipette. Record the volume of the cell culture.
- 8.7.2.4. Re-mount the head plate on top of the vessel and fasten with the six mill nuts finger-tight.

8.7.3. *Pre-clean the bioreactor in place*

- 8.7.3.1. Loosen the six mill nuts that fasten the head plate on the bioreactor vessel and remove the head plate
- 8.7.3.2. Fill the bioreactor with a working volume of 0.1M NaOH solution (2.4 liters for a 3-liter bioreactor).

SOP: Applikon ez-Control Bioreactor Controller Operation

- 8.7.3.3. Re-mount the head plate on top of the vessel and fasten with the six mill nuts finger-tight
- 8.7.3.4. Connect the stirrer motor per section 8.3.6.
- 8.7.3.5. Activate the stirrer at 250 RPM for 30 minutes. Visual check for dissolution of foam, debris and other contamination in the bioreactor.
- 8.7.3.6. Stop the stirrer. Lift the stirrer motor from the bioreactor head plate and set the motor aside.
- 8.7.3.7. Loosen the six mill nuts that fasten the head plate on the bioreactor vessel and remove the head plate
- 8.7.3.8. Drain the bioreactor.

8.7.4. Disassemble the bioreactor and clean all parts

- 8.7.4.1. Remove all tubing and gas filters from the bioreactor head plate assembly.
- 8.7.4.2. Remove the septum from the head plate.
- 8.7.4.3. Remove the air outlet condenser from the head plate and disassemble the condenser for cleaning.
- 8.7.4.4. Remove the head plate from the bioreactor vessel.
- 8.7.4.5. Clean all parts carefully and thoroughly using a small soft bristle brush (e.g. tooth brush) and a dilute laboratory glassware cleaner. Rinse thoroughly with MilliQ water and spray with 70% IPA and place on paper towels on a lab bench to dry.
- 8.7.4.6. Let dry all the parts.

8.7.5. Clean the porous sparger tip (optional)

Depending on the type of medium that is used (presence of proteins and/or peptides), the porous sparger tip may require a special cleaning procedure.

- 8.7.5.1. Remove the sparger tip from the air inlet pipe.
- 8.7.5.2. Soak the sparger overnight in a solution of 10 mg/mL pepsin / 0.01M HCl.
- 8.7.5.3. Use ultrasonic cleaning with water and/or ethanol.
- 8.7.5.4. Replace the sparger tip onto the air inlet pipe.

SOP: Applikon ez-Control Bioreactor Controller Operation

9. Attachments

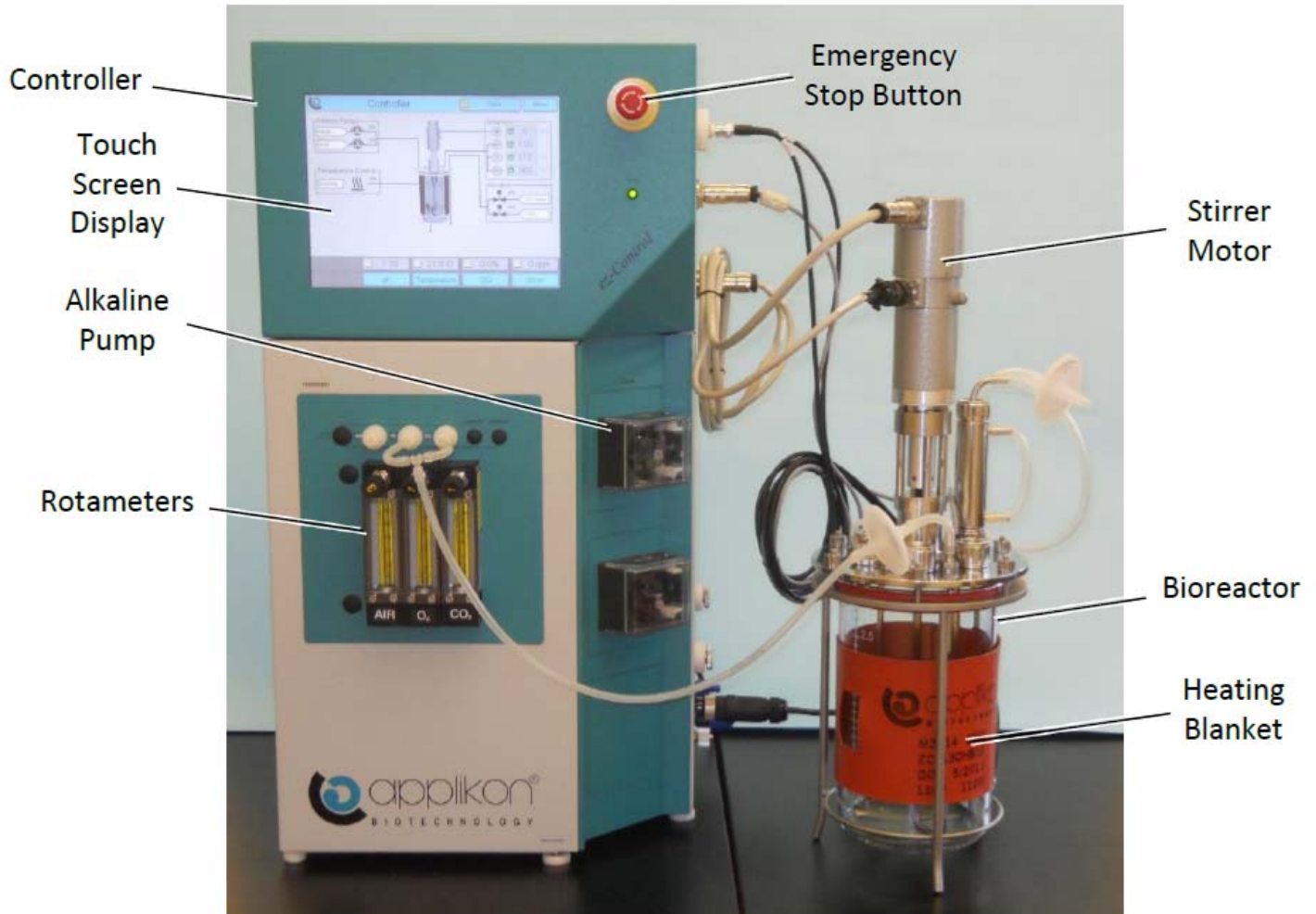


Figure 1. Applikon ez-Control Bioreactor Controller and Bioreactor

SOP: Applikon ez-Control Bioreactor Controller Operation

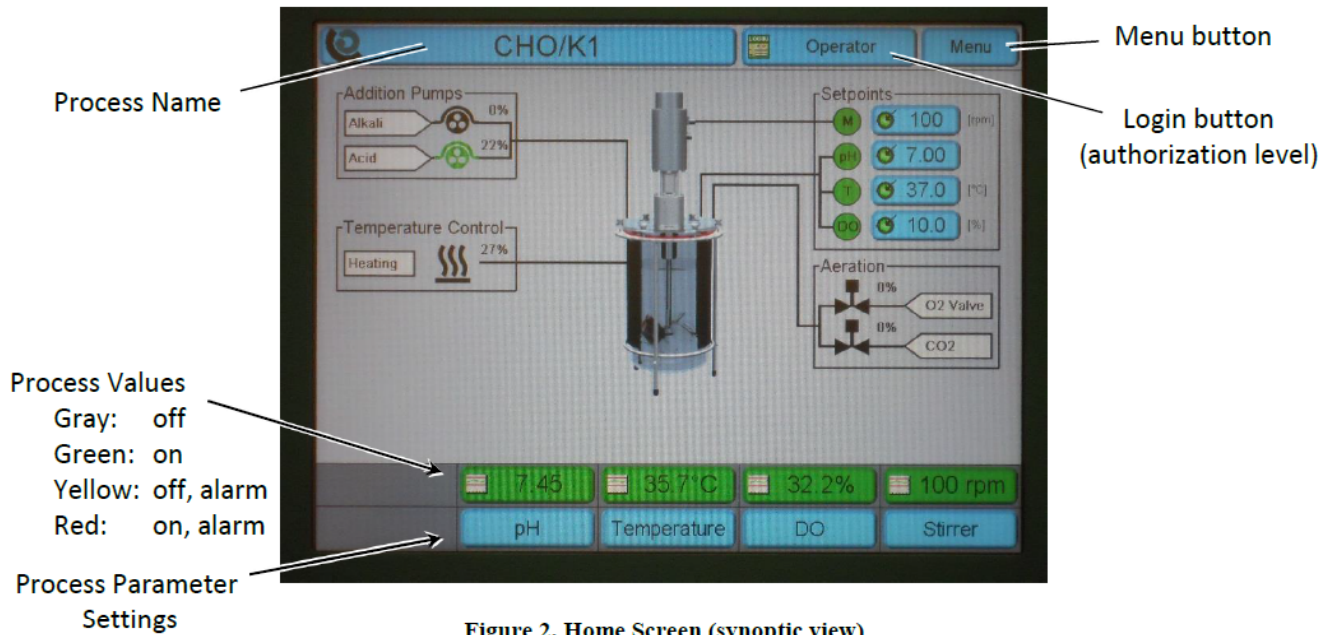


Figure 2. Home Screen (synoptic view)

Example 1. Navigating the Controller Screens to Login

Instructions	Explanation
<p>Home > Login (top middle) > Operator > Login Enter the Operator password: "0000"</p>	<p>On the Home screen, touch the Login button (top middle). The display changes to the Access Control screen.</p> <p>On the Access Control screen, touch the button Operator (left side). The display changes to the Operator screen.</p> <p>On the Operator screen, touch the button Login. The display changes to a keyboard and prompts for the Operator password.</p> <p>Enter the Operator password: "0000" Then touch the button Enter. The display changes back to the Home screen.</p>

SOP: Applikon ez-Control Bioreactor Controller Operation

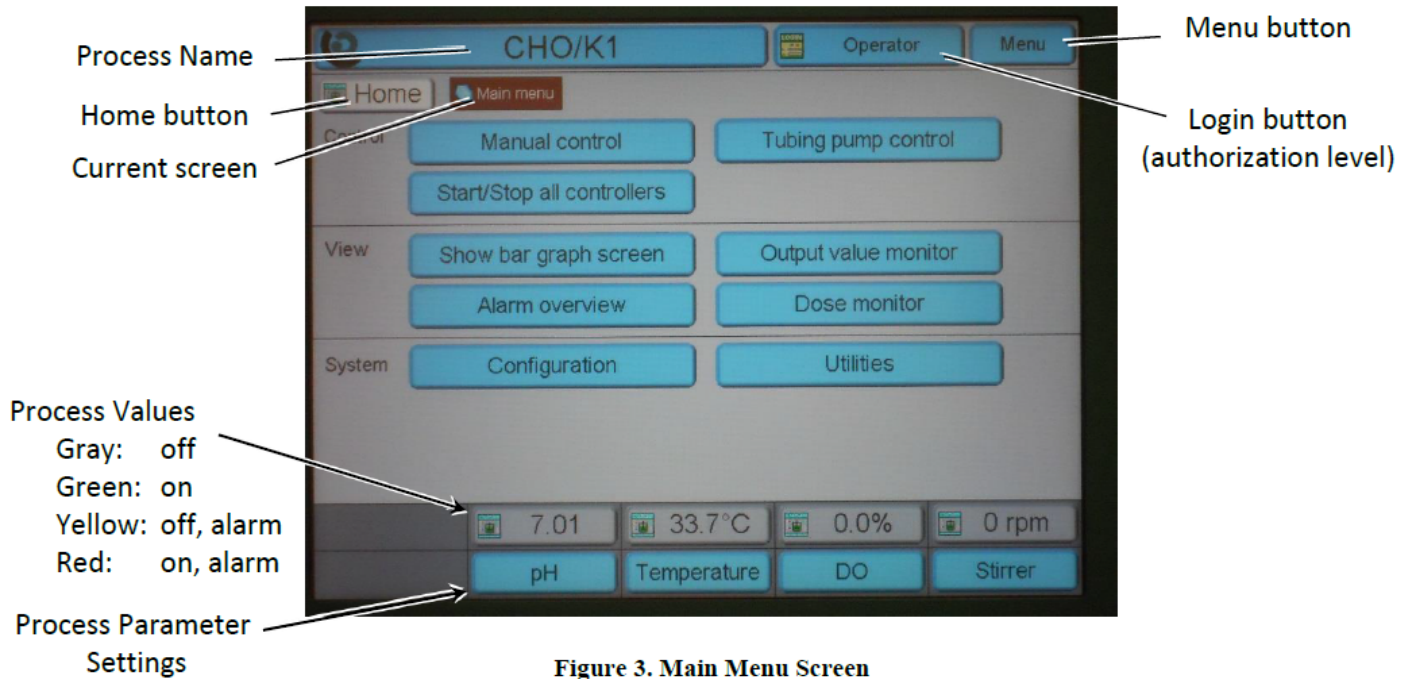


Figure 3. Main Menu Screen

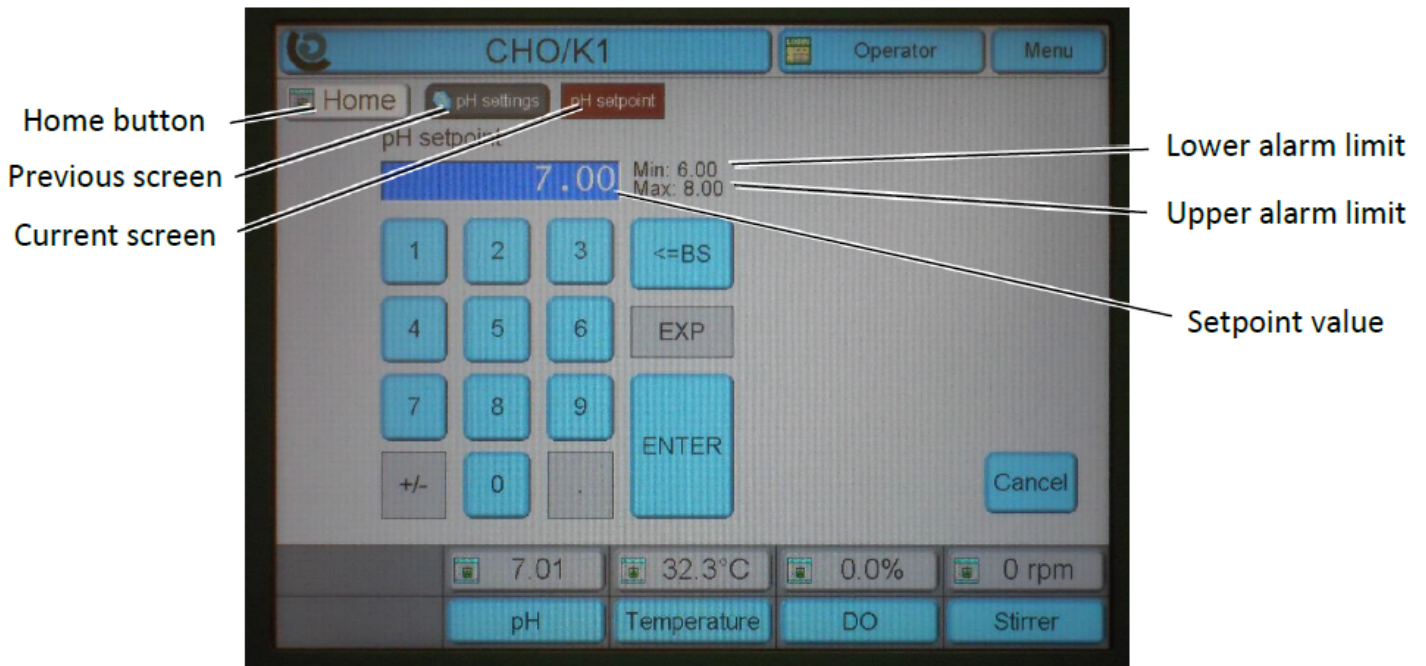


Figure 4. pH Setpoint Screen

SOP: Applikon ez-Control Bioreactor Controller Operation

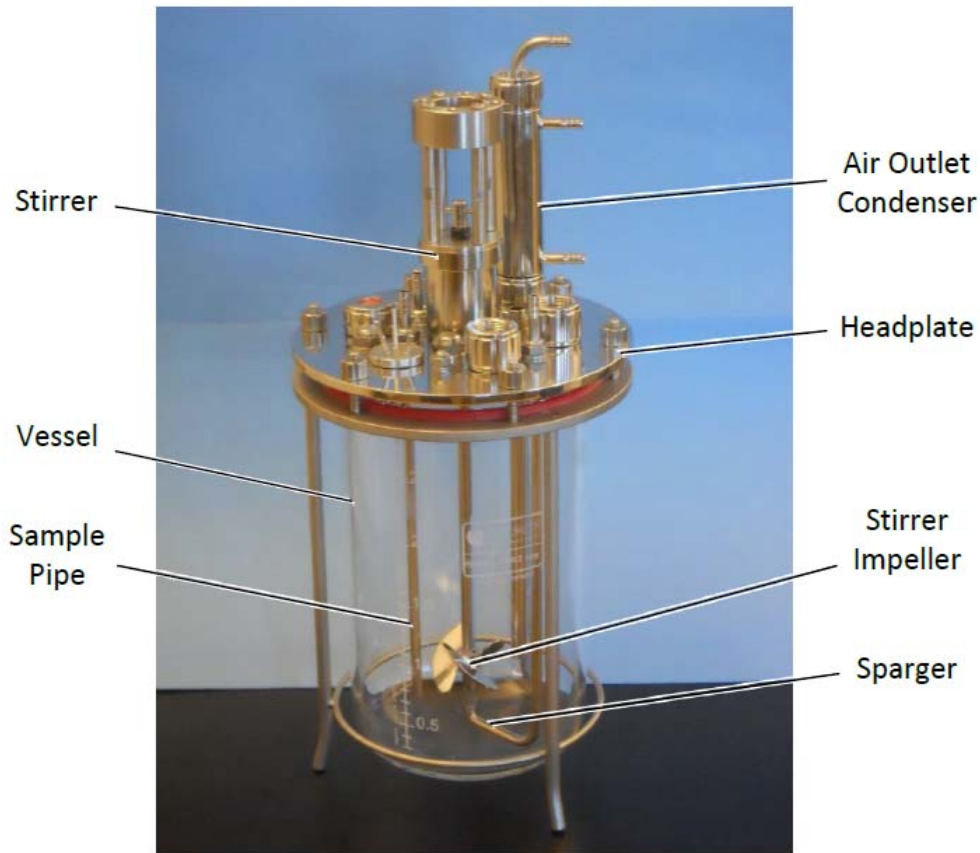


Figure 5. Applikon 3-Liter Glass Autoclavable Bioreactor

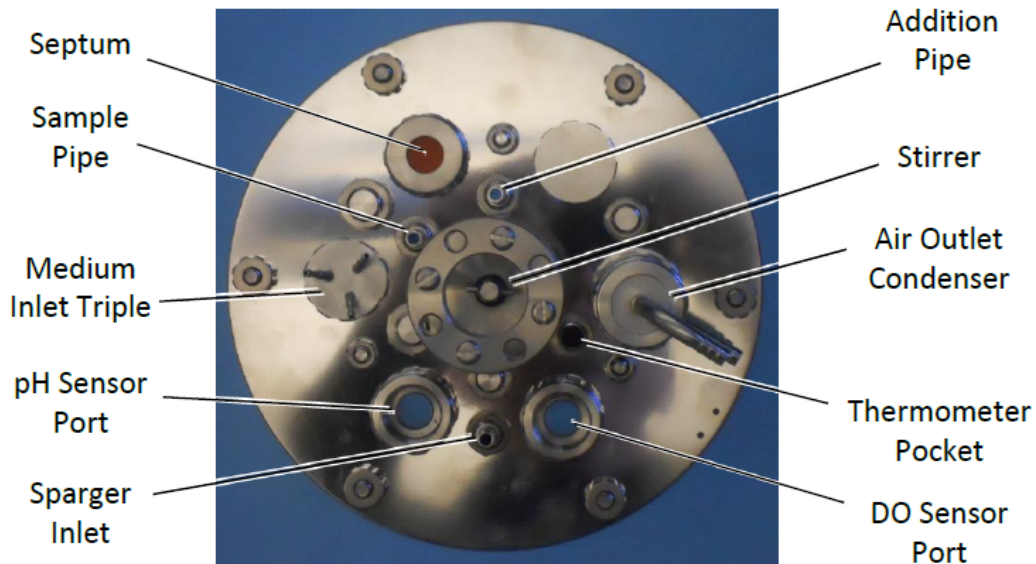


Figure 6. Bioreactor Head Plate (top view, unassembled)

SOP: Applikon ez-Control Bioreactor Controller Operation



Figure 7. Liquid Addition Bottle

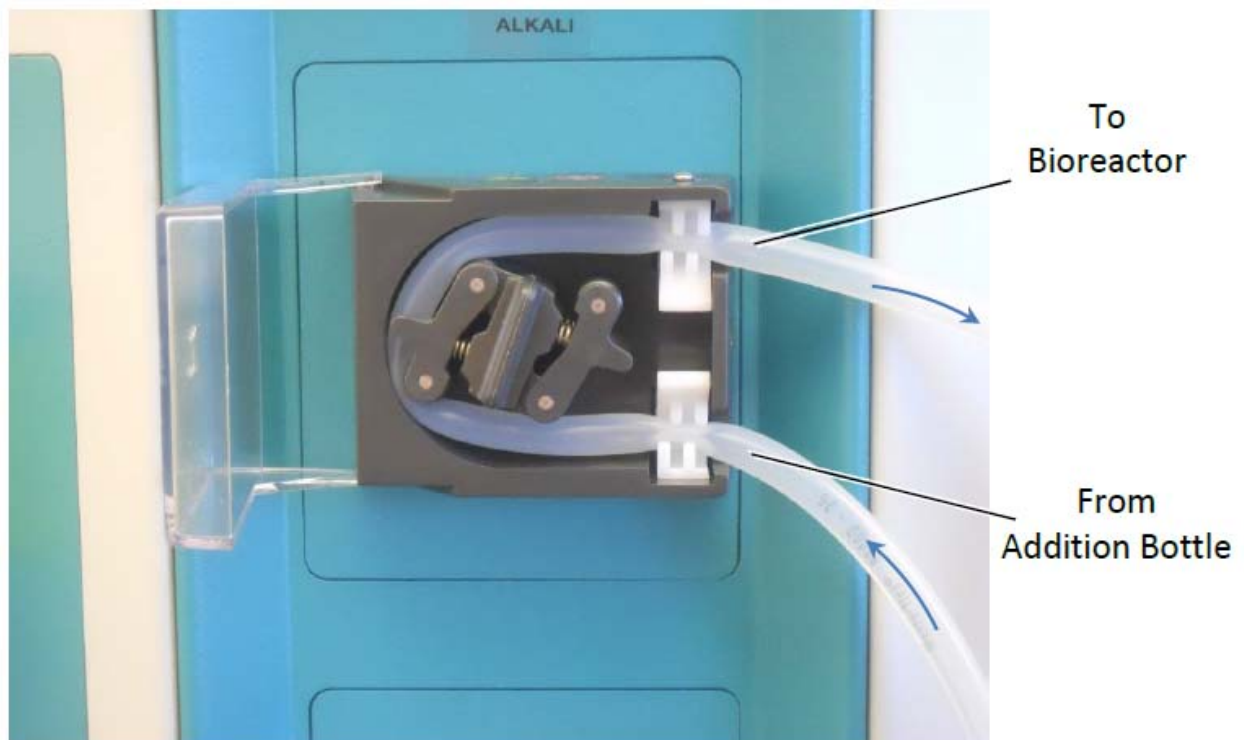


Figure 8. Peristaltic Pump (Alkaline Pump)

SOP: Applikon ez-Control Bioreactor Controller Operation

10. History

<i>Revision Number</i>	<i>Effective Date</i>	<i>Preparer</i>	<i>Description of Change</i>
0	20DEC13	John Buford	Initial Release
1	16SEP14	Jason McMillan	Various fixes and adjustments
2	10JULY17	Hetal Doshi	Added Details
3	25 JULY21	Hetal Doshi	Added sampling using Sample Bottle